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BOSTON UNIVERSITY
GRADUATE SCHOOL

Thesis

THE RESPONSE OF THE UTERINE SMOOTH MUSCLE OF THE RAT TO HISTAMINE
AND IN ANAPHYLACTIC SHOCK.

by

Caroline tum Suden
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A.M., Columbia University, 1927

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INTRODUCTION

The extraordinary resistance of the rat to certain drugs, toxins and in particular to histamine and to anaphylactic shock is at present a familiar fact. This resistance, however, can be decreased by removal of the adrenal glands. Lewis (1923) and Belding and Wyman (1926) demonstrated that double adrenalectomy increased the sensitivity of the rat to various substances, such as diphtheria toxin, cobra venom, curare, adrenalin, etc. Crivellari (1927), Marmorston-Gottesman and Gottesman (1928), Scott (1928), and Wyman (1928) showed that the same was true for histamine. In 1929 Wyman confirmed the only other work (Flashmann, 1925) on the increased susceptibility of the rat to anaphylactic shock after the removal of the adrenal glands. Previous to the relatively recent disclosure of the unusual tolerance of the rat to histamine, it was noted that this substance relaxed the rat uterus, pregnant or non-pregnant, in contradistinction to its effect on the uterus in other species. This was first reported by Guggenheim (1912) and corroborated by Fuhner (1913), Gunn and Gunn (1914), Longcope (1922), and Kelloway (1930). In anaphylactic shock, however, the findings of Parker and Parker (1924) and Kelloway (1930) indicated that the response of the rat uterus was undoubtedly one of contraction, although by no means as striking or as characteristic as that seen in the guinea pig. Longcope (1922) made a similar study but obtained only negative results.

Because of the striking similarity of the gross and pathological symptoms found in histamine and in anaphylactic shock (Mellanby, 1913; Dale, Laidlaw, 1919) and of the method for influencing resistance, it seemed advisable to compare again the responses of the uterus in histamine and in anaphylactic shock, in view of the difference in reaction. Reinvestigation of the normal rat uterus promised only further substantiation of the above results. Nevertheless, it seemed that a study of the doubly adrenalectomized rat, with and without cortical tissue, might produce some evidence to show that this exceptional behaviour of the rat non-striated muscle is not conclusive proof that the release of histamine is without influence on the anaphylactic syndrome of the rat as advocated by Kelloway.

HISTORY

Tolerance of the Normal Rat to Drugs and to Anaphylaxis. The work on drug susceptibility in the rat led to the disclosure of the unique action of histamine in this animal and also served as a background for the studies of the functions of the adrenal glands in relation to drug susceptibility, infections, etc. Moreover, attention was directed to a possible association of the underlying mechanism in drug reaction with the comparable situation found in anaphylaxis.

According to the customary expression in the literature for the last twenty years, the rat has been accorded the

reputation for "well known" resistance to drugs, poisons, toxins and bacterial infections. In fact, this phrase was employed by Gunn in 1912, but no specific source of information was given. Since this idea has been closely associated with the more recent findings on rat tolerance both in histamine and in anaphylactic shock it seemed worthwhile to obtain some information on the source of this opinion. The material found is given as an appendix to the present paper, since the drugs discussed have no direct bearing on the subject at hand, except that the opinion of Voegtlin and Dyer (1925) that tolerance is encountered only to those drugs which effect the non-striated muscle of the rat, is in some measure upheld.

The resistance of the rat to histamine presents one of the more striking and indisputable examples of the phenomenon of congenital tolerance. This fact was disclosed only comparatively recently by the work of Voegtlin and Dyer. Being surprised to find that the rat could withstand such large doses, they proceeded to make a study of the resistance of the rat to drugs. They found that the intravenous M. L. D. of histamine was around 900 mgm. per kilogram. Previous to this work, Longcope in 1922 reported that although intravenous injections of 10 mg to 15 mgm. per 100 gms. in the rat caused collapse, recovery nevertheless ensued. In one case only 30 mgm. per 100 gms. caused death. In 1927 Crivellari found that 500 mgm. to 700 mgm. per kilogram intravenously was fatal. In 1928 Marmorston-Gottesman and Gottesman worked out the

M. L. D. in two series, one by intraperitoneal and the other by intravenous injections. By the intraperitoneal route they found that 1600 mgm. per kilo caused death; but they obtained only one death by intravenous injection, and that with 2000 mgm. per kilo. They attributed their higher figures to the age of the rats, which was about six months. Because of the expensiveness of the drug Scott (1928) did not determine the dose exactly, but he found that intraperitoneal injections of 110 mgm. to 130 mgm. per 100 gms. were not fatal. For the same reason Wyman ascertained in a series of 15 rats, 1-2 months old, that recovery would take place after intraperitoneal injections of 100 mgm. per 100 gm. body weight. Rachemann (1931)) states that 125 mgm. per 100 gms. intraperitoneally produced no symptoms. In 1929 Schmidt and Stahelein reviewed the work on the relative toxicity of histamine for the different species. Using histamine chlorhydrate intravenously they found that 230 mgm. to 270 mgm. per kilo was fatal for the rat. They believed that the figures given by Crivellari to be nearer the true M. L. D. for ergamine acid phosphate, which was the substance used by all the other investigators. The following is a list compiled by Schmidt and Stahelein showing the variation in susceptibility of several species, judging from the lethal dose expressed in mgm. per kilogram of animal; guinea pig, 0.30; rabbit, 1.0-3.0; pigeon, 1.5; dog, 3.0; mouse, 250.0; rat, 300.0; frog, 1700.0.

Since Cannon (1918, 1919, 1923) advanced the theory that

the causal agent in traumatic shock may be the liberation from the injured tissue of ~~the~~ substance or substances similar to histamine, it is interesting to note here that Voegtlin and Dyer were unable to produce traumatic shock in rats, even after extensive injury to both hind limbs. This seems to be the only published work on this subject. Wyman (unpublished) has also experimented with traumatization of rats, but the results so far have proved unsatisfactory.

Between 1903 and 1910 the attention of immunologists was drawn to the fact that rats were refractory to anaphylaxis. At the Microbiological Conference at Berlin in May, 1910, Uhlenheuth convincingly affirmed that rats and mice were insusceptible to shock as seen from the results of an extensive investigation of the response of numerous species to a variety of antigens. This work was carried on in conjunction with Haendel, Weidanz and Steffenhagen. In 1909 Trommsdorf, also in his laboratory, failed to obtain any positive signs of shock in rats and mice. In 1910, Galli-Valerio, using *M. rattus*, *M. decumanus*, and *M. musculus* also failed. The only work not in accord with the above findings was that of Arthus in 1903 and Rosenau and Anderson in 1906. In both instances the reports merely listed the rat as one of the species in which they had observed symptoms of shock, no other data being recorded. With respect to mice, which are frequently very similar in their reactions to rats, Sachs claimed at this same meeting that the above generalization did not hold --

Trommsdorf, Freidmann, Frey, and Deorr to the contrary. However, later in 1913, Sarnoski stated that a fatal outcome could be obtained in mice by intravenous or intraperitoneal sensitization and injection of the shocking dose by the same route if huge amounts of antigen were employed, and allowing only 10-12 days for the incubation period.

The work of Novy and de Kruif (1917) on anaphylatoxin again directed attention to this question. A non-specific lethal response was obtained in sensitized rats on the re-injection of distilled water or antigen greatly diluted with distilled water. This reaction did not obtain in non-sensitized rats. Experiments with pure egg albumin, horse serum, beef serum and rabbit serum as antigen, following the customary procedure were negative. Furthermore, De Kruif found that the rat was 100 times less susceptible to anaphylatoxin than the guinea pig; three times as much agar-sol-gel and about ten times as much peptone were required to effect the rat.

On the basis of the foregoing work, Longcope (1922) chose the rat for his study on insusceptibility to sensitization and to anaphylactic shock. He found that the results of the injections of 1.0 cc. test dose of horse serum, 21-44 days after sensitization by subcutaneous, intravenous, subdural and intraperitoneal routes were practically negative and in no case fatal. However, between the 4th and 9th day after sensitization the precipitin titre for horse serum was fairly high in contradistinction to the condition found in the guinea pig, which is easily sensitized and shocked. He also was unable to confer

passive sensitization upon guinea pigs and rabbits with sensitized rat serum. He concluded therefore, that the formation of precipitin and anaphylactin were not parallel processes. He further stated that as the rat fails to form the latter complex, it does not behave the same immunologically as do other animals and is therefore invulnerable to the antigen-antibody reaction or to "what it is that elicits shock". Longcope's work on precipitin formation in the rat was confirmed later by the work of Spain and Grove in 1925.

In subsequent discussions of Longcope's paper it was pointed out that his completely negative results may be attributed to the fact that he did not make any tests for shock within the 21 day incubation period, (Flashman, 1925). In 1924, Parker and Parker pursued a similar study of the rat and claimed that they were able to procure positive evidence of anaphylaxis. In a series of 18 cases sensitized to horse serum and a second series of 28 rats sensitized to sheep serum, they observed generally slight with some severe symptoms on the re-injection of the homologous antigen after an interval of 9 to 14 days. Three deaths were recorded for the series in which sheep serum was employed. Passive sensitization with anti-horse sheep serum produced only slight symptoms. In three cases they tested for shock by the Dale method and obtained moderate uterine stimulation in only 2 out of the 3 tests. The comment of Zinsser, in his third edition of *Infection and Resistance*, was that the symptoms were mild. Seegal and

Khorazo (1929) also made an interesting observation on the above work in connection with their problem on the effect of diet upon the anaphylactic response of the rat. They showed that rats fed on a diet rich in vitamins consistently developed slight or no symptoms, whereas those fed on a diet of bread and water constantly exhibited definite signs of shock, although they rarely succumbed. The results were in harmony with the findings of Wedgewood and Grant (1924), who stated that rats deficient in vitamin B became susceptible to shock. Seegal and Khorazo investigated this point because they believed that Parker and Parker's positive results were due to the above condition, since contemporary reports from the animal-house indicated that the rats were maintained on a diet consisting chiefly of bread and water.

Since Parker and Parker were the only ones to achieve success, their method has been employed by subsequent investigators. Ebert in 1927 attempted to confirm their results by the same method. Out of 29 normal sensitized rats only one showed definite symptoms such as described by Parker and Parker. Ebert raised the question as to why only these of all the group of immunologists and others experienced authorities were able to produce anaphylaxis in the rat. The answer may be found partly in the results of Seegal and Khorazo, or the following point brought up by Schmidt and Stähelein (1929) may account for this event in some measure. They found that the rat, being extremely

(1) The first of these is the fact that the
 water is not in contact with the air, but is
 contained in a closed vessel, and the only
 means of escape is by the action of the
 steam, which is forced out of the vessel
 by the action of the heat. This is the
 principle of the steam engine, and it is
 the same principle which is used in the
 construction of the steam boiler. The
 water is heated by the action of the
 steam, and the heat is transferred to
 the water by the action of the steam.
 The water is then forced out of the
 vessel by the action of the steam, and
 the steam is condensed by the action of
 the water, and the cycle is repeated.
 This is the principle of the steam
 engine, and it is the same principle
 which is used in the construction of
 the steam boiler. The water is heated
 by the action of the steam, and the
 heat is transferred to the water by
 the action of the steam. The water is
 then forced out of the vessel by the
 action of the steam, and the steam is
 condensed by the action of the water,

resistant, required unusually large amounts of antigen for sensitization and shock. In relation to the earlier dispute on the response of mice to anaphylaxis they stated that the same held true for the latter, but that mice were more easily shocked than rats. Wyman (1929) obtained only slight or moderate symptoms in 19 cases out of a series of 29 normal rats. The remaining 10 rats showed no effects of the test dose. In 1930 Kelloway took up the question of the relation of histamine to anaphylaxis by making a study of the smooth muscle reaction of the rat. He also found that the rat was comparatively insensitive to this phenomenon by active sensitization. Furthermore, he obtained only two positive responses out of 15 tested. With passive sensitization, the uterine tests regularly indicated a positive reaction although the rats (intact) were apparently only irregularly reactive.

The failures of Friedberger and Seidenhantz (1911), Opie (1924) and Nordman (1931) to obtain the Arthus phenomenon should be mentioned to complete this account on the resistance of the rat to anaphylaxis. To summarize the results reported, one may conclude that the rat has been found to be consistently insensitive, judging from outward manifestations. This may include the work of Parker and Parker if one takes into consideration the opinion of Zinsser. Their detailed search for and study of symptoms, in conjunction with the findings of Kelloway, indicate that this phenomenon takes place in the rat but is ineffective in producing constant, pronounced,

outward manifestations. There is undoubtedly a wide individual variation in sensitivity, which they noted in older rats, and which may have, along with other factors as diet, age, technique, dosage, contributed some reason for the total failure of others to find any indications of anaphylaxis.

Susceptibility of the rat after double adrenalectomy. Adrenal physiology received an appreciable impetus from the work of Lewis in 1920-1923 on the increased susceptibility of the rat to poisons and drugs after double adrenalectomy. This particular aspect of the field had been almost neglected up to this time, although in the latter part of the nineteenth century French and Italian physiologists had given it considerable attention. Recent authorities generally claim that the vast amount of early work led only to contradiction and confusion. From a survey of this literature Lewis decided that the subject could well be reinvestigated and amplified by a more methodical study with adequate controls.

The study of adrenal extirpation in rats was in progress by 1856 according to the reference of Artundo (1927) to Phillieaux (1856) and Harley (1859) who demonstrated that rats could survive such operations. In 1895 Boinet made a similar communication, and in 1896 and 1897 he demonstrated that the toxic effects of muscle extracts, ouabain and neurine were greatly increased for rats from which the adrenals had been excised, ligated or cauterized with silver nitrate, iodine, or ferric chloride. Langlois and Charrin (1896)

published a paper of a similar nature. Later, Schwarz (1910) and Kahn and Starkenstein (1911) found the same to be true for adrenalin. In 1921 Lewis confirmed this finding and also determined that the adrenalectomized rat was more susceptible to atropin, veratrin, digitoxin, cobra venom, and curare. Of the convulsant poisons, picrotoxin and strychnine, he observed no alteration in toxicity. In 1923 he added to the list insulin, papaverine and codeine. In 1921 he pointed out that 1/400 M. L. D. of morphine for the normal rat caused death in some recently adrenalectomized animals. This observation precipitated a long lasting dispute with Rogoff and associates (1925, 1927) who were unable to confirm his results in their test on three or four rats. Lewis repeated his work on morphine in 1923, and again later in 1926 in conjunction with Torino. They found that 0.04 mgm. per gram of the chlorhydrate was fatal to rats up to two weeks after operation whereas the M. L. D. for normal rats was at least 0.4 mgm. per gram body weight. Moreover, this difference gradually diminished according to the length of time which had elapsed between operation and injection, which they attributed to the development of accessory cortical tissue. In 1923 Scott had also demonstrated that after adrenalectomy three-fifths to one-tenth of the M. L. D. of morphine for normal rats was fatal ⁱⁿ to a large percentage of cases.

In 1926 Crivellari found that effect of operation decreased the lethal dose of acetonitril from 5.0 mgm. per gm.

to 0.05 mgm. per gm. (normal toxic dose of 5.0 mgm. per gm. confirmed by Hunt and Seidel); of cyanide, from 11.0 mgm. to 8.0 mgm. per kilogram; of nicotine, from 27.5 mgm. to 17.5 mgm. per kilogram; and of histamine, from 500 mgm. to 40 mgm. per kilogram. This latter example, along with Voegtlin and Dyer's previous communication on the resistance of the rat to histamine and the increasing interest in the physiological importance of this substance, stimulated Scott (1928), Wyman (1928) and Marmorston-Gottesman and Gottesman (1928) to further study of the relative role of adrenal cortex and medulla in combating histamine shock. In so doing they established and confirmed the magnitude of the toxic dose for normal rats and the marked increase in toxicity after double adrenalectomy.

Other investigators took up the study along the lines of bacterial infection and intoxication. Scott (1924) observed that an injection of a given amount of killed streptococci which induced only slight symptoms in normals was 100% fatal to adrenalectomized rats in seven hours. Subsequent work by Jaffé and Marine (1924), Jaffé (1926) and Molinelli (1926) confirmed Scott. With respect to diphtheria toxin, Lewis (1923) found that 100 M. L. D.s for the guinea pig was fatal for the adrenalectomized rat, but Belding and Wyman (1926) estimated that the normal rat was only $2\frac{1}{2}$ times as resistant as the operated animal. These findings confirmed the earlier claims of Oppenheim and Loepe (1901) and Lusena (1902). Recently Steinbach (1928) has been able to confer both avian

and bovine tuberculosis on rats by removal of the adrenals. Such animals, however, are immune to the human strain.

Very little work has been done on the increased susceptibility of rats to anaphylaxis after such operations. Kepinow (1922), in his experiments of this nature on guinea pigs, obtained results which supported such an assumption. In 1925 Flashmann extended the work to rats. Despite the difficulties arising from respiratory infections and operative technique, he was able to secure a limited series of significant results. A comparison of the adrenalectomized with that of the normal rat showed that in the former the manifestations of shock were more severe and in some cases unquestionably fatal. Wyman (1929) met with better success. Normal rats exhibited only slight or no symptoms. Moderate to fatal effects were encountered in at least 65 percent of the operated rats which had been sensitized prior to or subsequent to extirpation of the glands. In rats having accessory cortical, but no demonstrable chromaffin tissue the mortality and severity of the reaction ran equally high.

Although there seems to be no other reference in the literature to this type of diminished resistance in the rat, the observations are confirmatory of and in harmony with the facts just reviewed on altered drug toxicity after adrenalectomy and the change in resistance in anaphylaxis and drugs from dietary deficiencies. The peculiarities of the rat, just surveyed, namely the natural resistance to drugs, esp-

ecially histamine, and to anaphylaxis, together with the decrease in tolerance in general after removal of the adrenals has not only stimulated physiological interest in the functions of the glands but also in the cause or mechanism of anaphylactic shock.

The possible role of histamine in anaphylactic shock. Before reviewing the subject of the possible role of histamine in anaphylaxis it seems advisable to recall the fundamental characteristics of this phenomenon.

The term 'anaphylaxis' is restricted to a particular type of hypersensitivity, a condition in which the reinjection of a previously harmless, soluble, antigenic protein after a suitable lapse of time incurs a profound physiological disturbance, the manifestations of which are the same regardless of the nature of the antigen used, but peculiar to the species of the reacting animal. In defining a condition as anaphylactic, the consensus of opinion adheres to the criteria outlined by Wells (1922) with some slight modifications. They are as follows:

1. The observed toxicity of the injected soluble protein material must depend upon the development of immunity of the animal and should not produce similar symptoms on the first injection.

2. The symptoms must be the same for any antigen used but must differ characteristically with the various species of animal.

3. It should be possible to demonstrate passive sensi-

tization.

4. It should be possible to demonstrate contraction of the isolated non-striated muscle.

5. It should be possible to demonstrate desensitization.

The sudden pronounced fall in rectal temperature discovered by Pfeiffer, and the lowered coagulability of the blood, pointed out by Friedberger, are considered by some as essential diagnostic features. Wells also adds that the exclusion of the possibility of embolism and thrombosis as well as the use of atropin and adrenalin to alleviate the symptoms, are important points to be taken into account.

Other forms of hypersensitivity, e. g., to drugs, physical agents, poisons, etc., which closely resemble anaphylaxis but do not fulfill all the above requirements, are classified as allergic phenomena. Because of the close resemblance of these conditions, the probability of a similar underlying mechanism has been emphasized by many authorities, but as yet there is no conclusive evidence on this matter.

With the rapid advancement in the understanding of this phenomenon, especially by the well-known experiments of Schultz, Dale, Manwaring, Pearce, Eisenbrey, Coca, Weil, etc., one vital point has been indisputably established, namely that the site of the reaction is primarily a cellular one and not as was originally supposed within the blood stream. Consequently the anaphylatoxin theory of Friedberger and the modifications or elaborations of it by Vaughn,

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Jobling and Peterson, has been found inadequate as an explanation, chiefly on the grounds that the readily produced toxicity of the blood which they showed to occur through protein digestion by the action of antigen, antibody and complement, enzymes, or numerous inert, non-specific substances, would involve not only the participation of complement, and a humoral site for the reaction, but offered no reason for the stimulation of excised smooth muscle, the time requirement for passive sensitization, and the inhibiting influence of circulating antibodies. In fact, Wells lists thirteen objections to this theory, but he states:

The fact that slight disturbances in the equilibrium of plasma colloids renders them highly toxic is an outstanding fact, and it becomes easily understandable that a similar alteration in colloid equilibrium within the cell protoplasm may produce equally profound intoxication of the cell, so that at present there is a growing tendency to seek an explanation of anaphylaxis in the domain of colloidal chemistry.

Zinsser in discussing this subject calls attention to some exceptions to the above objections to the anaphylatoxin theory, based on experiments by reliable observers-La Maire, Friedmann, Richet, Biedl and Kraus, Brest, Curd and Scott. Profound shock, resembling anaphylaxis in every way, was produced by the simultaneous injections of antigen and antibody into the blood stream or some analogous procedure.

Hence, Zinsser concluded that under certain special conditions the meeting of antigen and antibody in the blood stream may cause injury, and may account for the Arthus and anaphylactoid phenomena.

On the basis of the classical smooth muscle experiments of Schultz (1910) and Dale (1912), Wells correlated the outstanding symptoms displayed by different animals with the variations in the distribution of non-striated muscle. The bronchospasm of the guinea pig, the pulmonary, arterial constriction of the rabbit, and the gastro-intestinal disturbances of the dog are as typical for these species as is the comparative predominance of the smooth muscle fibres found in these respective situations, locating the basic disturbance in or on this tissue. On the other hand, in all species the fall in blood pressure and accompanying oedema is regarded as a constant, primary lesion. This latter manifestation of shock clearly involves an alteration of the reticulo-endothelium, particularly in the direction of an increased capillary permeability. It merits particular concern as the fundamental change responsible for the shock syndrome, especially in the dog and in the rat. Many recent experiments have been devised to lend support to the opinion that this is the dominant factor, whether it is the site of the primary reaction or only the major injury resulting from the liberation of toxic substances from the meeting of antigen and cellular antibody elsewhere. This contention is based upon the fact that Vanucci (1924) and Gay and Clark (1924) demonstrated

that by blocking the endothelial cells with trypan blue antibody formation was inhibited. Many others have since worked along the same line and claim that such substances as India ink, congo red, iron oxide, saccharose, and bacteria also protect against shock. (Peterson and Levison, 1923; Moldovan and Zolog, 1923; Klinge 1927; Ehmer and Hammerschmidt, 1928; Nikolaeff and Goldberg, 1930; Haendel, 1930). It is interesting to note that the course of peptone and histamine shock is also similarly altered, (Haendel, 1930; Moldovan and Zolog, 1923). But the strongest evidence for the involvement of the reticulo-endothelial system in anaphylaxis is derived from the experiments of Manwaring (1923), who maintains that the crucial feature of shock lies in the increased permeability of the capillaries, all other reactions being secondary. On perfusion of sensitized isolated dog lung with saline containing antigen (horse serum) he obtained a 75% reduction in the rate of perfusion flow, a non-collapse of the lung and an unmistakable escape of the fluid into the tissues. He has also shown that on removal of the liver from a sensitized dog the injection of antigen failed to elicit any signs of shock but, if a sensitized liver were transplanted into a normal dog, all the signs of shock developed on the injection of antigen. Furthermore, Manwaring and his associates (1917-1923) have shown that the liver undoubtedly acts on antigen, for perfusion of antigen through the liver of a sensitized guinea pig lost its toxicity for sensitized guinea pig lung. Such did

not occur with normal liver perfusion. In this connection Fall (1918) stated that larger doses of antigen were needed to produce shock when injected into the portal vein than into the jugular. Weil also found that the release of anti-coagulant substances was dependent upon the perfusion of antigen through the sensitized liver, presumably due to cell injury as is the case with poisons. Likewise, the Arthus phenomenon and skin reactions of man are definitely indicative of vascular injury. Another feature, the increased flow and protein concentration of the thoracic lymph (Peterson and Levison, 1923) is the direct outcome of an increased capillary permeability.

Nevertheless, despite all this evidence in support of Manwaring's view in which he postulates that the altered permeability of the capillaries, owing to the liberation of a toxic, depressor substance by the liver, is the dominant physiological change in anaphylaxis, the objections have yet to be overcome that it does not adequately account for the local anaphylactic reactions and for the experiments with isolated organs. From the report of Dragstedt and Gebauer-Feulnegg at the Federation of American Societies for Experimental Biology, Philadelphia, 1932, it seemed that these difficulties may be met in some measure, as they were able to prove the presence of a depressor substance in the blood from the vena cava and in the thoracic lymph which activated the isolated guinea pig uterus. Since then they have been able

to report that this substance can be isolated in fairly pure form, though not in pure crystalline form. It has been tested chemically and physiologically, and so far the results show that it consistently conforms to the reactions produced by histamine. Practically, all that remains to be done is to ascertain the molecular constitution, which demands a larger quantity of the material than they have been able to obtain. The work is still in progress.

The symptoms of anaphylaxis, the conditions stressed by Manwaring, and the reactions of this latest "hypothetical" material strongly suggest that the identity of this toxic agent may be histamine or the H-substance suggested by Sir Thomas Lewis (1927) from his studies on human allergy. In any case, if the Dragstedt, Gebauer-Feulnegg substance can be proven to be a specific, demonstrable entity, in the dog as well as in other species, then one of the greatest objections to the theory that histamine or a like substance plays a role in anaphylaxis will have been overcome.

From the foregoing survey it is easily seen that, despite the periodic criticism and rejection of the suggestion concerning the participation of a toxic substance in anaphylaxis, such an idea continues to insinuate itself into the picture as more light is thrown upon the probable significance of histamine in physiological processes.

Histamine was first isolated by Yoshimura in 1909 from putrid soy beans. In 1910 Barger and Dale isolated and identified it in ergot. From their earlier observations of its

pharmacological effects they called attention to the possible association of histamine or a similar substance with anaphylaxis. In 1911 Barger and Dale were the first to isolate it from an animal source, the intestinal mucosa of the guinea pig, and in 1912 Dale and Laidlaw demonstrated the similarity of these two responses on the surviving uterine strip of the guinea pig. In 1919 Abel and Kubota determined its presence in the extract of the posterior lobe of the pituitary and attributed the oxytocic action of this material to the presence of histamine. As a result a dispute ensued with Hanke and Koesler (1920-1924), Dudley, Dale and others, not only on this specific function of the posterior lobe, but chiefly on the question of the absolute amount of histamine present in fresh, uncontaminated tissue and the effectiveness of such concentrations. In connection with this controversy, although Abel and Macht (1919) and Adler (1918) showed that there was some slight activation of the uterine strip of the mouse in very low concentrations, and that the rat smooth muscle behaved similarly but more faintly (Abel, 1919), the customary reaction obtained to moderate amounts was one of relaxation, as earlier reports had shown (Guggenheim, 1912; Fuhner, 1913). This fact assumed considerable diagnostic importance in determining the presence of the amine. Without further consideration of this point Abel advanced the theory that the formation of histamine was the most probable explanation of the cause of anaphylactic, peptone, and hista-

mine shock as well as intestinal obstruction, because of the parallelism seen in the clinical syndromes of these conditions and the possibility that such a substance might be easily and explosively liberated from the tissue proteins in general. But it was not until 1927 that Best, Dudley, Dale and Thorpe proved conclusively that histamine was a constituent of perfectly fresh normal tissue, a derivative of histidine, which is in turn an amino acid component of every known complete protein.

Prior to this latter contribution, Dale and Laidlaw (1919) again called attention to the resemblance of histamine shock to anaphylactic shock, in that both caused the contraction of smooth muscle, especially stimulation of the excised guinea pig uterine strip, a peripheral dilatation of the capillaries and fall of the blood pressure, and the cutaneous reactions seen in man. They favored, however, an explanation based on the assumption of a protoplasmic colloidal disturbance rather than the formation of "anaphylatoxins". In the Second Herter Lecture (1920) Dale felt it necessary to reiterate that he had not expressed the idea that histamine was the cause of shock, as others had so misinterpreted his paper in 1919, but stated that he thought it possible that the liberation of histamine or other substances has an indirect effect in shock, and more particularly in traumatic shock.

Smith (1920) opposed the histamine idea for several

reasons based on his study of a comparison of histamine shock with anaphylaxis. The points of difference were, namely: that in guinea pigs and rabbits desensitization was not obtainable with histamine, and that the desensitized surviving muscle responded characteristically to the drug; that the fall in temperature and the decreased coagulability of the blood did not occur on injection of histamine; and that quinine augmented anaphylactic shock, but failed to do likewise in histamine shock. Except for the difference in the reaction of the rat uterus to both conditions, these four points are widely accepted as fairly conclusive evidence, although conflicting reports can be found on the matter of the fall in temperature and the lessened coagulability of the blood, depending upon the species under observation. In regard to quinine augmentation, nothing further seems to have been done, but according to pharmacological data on this drug it seems to be well established that quinine may accelerate biological processes in small doses, but certainly acts as a protoplasmic poison in larger quantities.

In 1921 Manwaring reasserted his previous opinion based on observations of dehepatectomized dogs, namely that the liberation of a depressor substance from the liver as a secondary process was the dominant contributing factor in the clinical manifestations of shock -- the initial, fundamental physiological mechanism not being identical in anaphylactic, histamine or peptone shock. The sudden fall of temperature ob-

served during shock did not occur in rabbits, although a gradual decline was observed during the experiments, undoubtedly due to operative procedures. In other species, as the dog, cat and guinea pig, Best (1931) has pointed out that others had obtained a drop in temperature after the injections of histamine. As for the change in coagulation time, Bally (1929) found that 13 rabbits out of a series of 19, showed an average decrease of 5 minutes and 20 seconds. Nevertheless, he concluded that anaphylactic shock could not be ascribed to a sudden development of either substance (peptone or histamine).

In 1930 Kelloway also investigated this theory from the standpoint of the rat uterine response, as the inhibiting effect of histamine had been well established, and as the only reports on the anaphylactic response of the uterus were those of Parker and Parker (3 cases) and Longcope's negative results. Confirming the above workers, he found that the reaction was hard to obtain and in no way as decisive as that of other animals. By passive sensitization he found that he could elicit a fairly constant, mild response from the uterus, and he considered the evidence sufficient to indicate that histamine played no role in anaphylaxis in the rat, and was certainly not associated with the anaphylactic stimulation of the smooth muscle. Yet the fact that there are a few sporadic reports in which one may find that a slight stimulation of the rat uterus has been obtained with

very small doses of histamine, indicates that there is not sufficient information on this subject. Katz, in the laboratory of Feldberg and Schilf (1929) also obtained a slight increase in the tone of the rat uterus, as did Abel and Macht (1919). Best, Dale, Dudley and Thorpe also reported that they had seen this same effect in a few instances when using one of their crude preparations. It is important to note that the rat uterus is considered generally to be rather insensitive to histamine, and that the response varies in degree. Voegtlin and Dyer (1925) determined that histamine in a dilution of 1:3,000,000 gave a definite picture of inhibition but subsequent workers have not been able to obtain this result with the same dilution. There is no question but that in moderate doses histamine is inhibitory. Abel has also pointed out that even in the guinea pig the uterus may be inhibited by large doses such as have been used on the rat and the mouse (Cow, 1919; Longcope, 1922), which are plainly paralyzing amounts.

On the other hand, an equally strong argument for the histamine theory has been presented by Kendall (1930) based on a study of the reaction of the surviving intestinal strips from sensitized guinea pigs. The addition of homologous antigen in fractionated doses to a fresh bath each time incited a contraction, the series of shortenings showing a gradual diminution in magnitude. If the previous stimulus was not washed out by renewal of the Tyrode's

solution, about ten times the original amount of antigen was required to elicit another or a further contraction. The stimulating influence of histamine acted in the same manner, but the response for a given concentration was always the same in normal, sensitized, or desensitized muscle. Large doses produced contracture, resembling the curve obtained with maximal amounts of antigen. In both cases the muscle could be brought back to a normal condition by washing out the source of stimulus. In addition, Kendall observed a lag before the uterus reacted to antigen; but to histamine it responded instantaneously. Kendall maintains that the above reactions are only explicable if the first step in anaphylactically induced contractions be due to protein cleavage followed by a liberation of a powerful smooth muscle contractant. The gradual exhaustion of antibody accounts for desensitization as well as for submaximal contractions. Consequently the argument against this theory on the grounds that desensitization does not occur in histamine shock he regards as poor logic.

In 1929 Dale again summarized the situation in his Croonian lectures. His opinion is that histamine or a similar substance is liberated as a secondary process in anaphylaxis together with other possible end-products. To these latter substances one could easily attribute the slight discrepancies between histamine and anaphylactic shock as exhibited by different species. According to Best and McHenry

(1931), such an explanation satisfactorily fits the known facts, but absolute proof still depends upon the development of a sensitive method for the estimation of histamine in the blood.

In addition to the work outlined above correlating the physiological reactions of animals in histamine and in anaphylactic shock, the study of human allergic phenomena has contributed many points which lend credence to this theory. The exact correspondence between an injection of histamine into the skin and the triple response obtained from allergic patients seemed to Lewis (1927) a striking illustration of the identity of the two, but due to lack of adequate and quantitative chemical proof he could not make any positive assertion that histamine liberation is the basis of allergic and asthmatic conditions. Yet since it has been found that so many unrelated agents such as heat, cold, pollen, foods, etc., produce a definite, identical syndrome in humans, it is difficult to discard such an hypothesis. Allergic patients have been found to be more sensitive to histamine than normals. Harris (1927) obtained an alcoholic extract of the skin which yielded a depressor substance very much like histamine. Kalk (1929) has shown that in dermographic patients he could obtain an increase in the acidity of the gastric juice by proper stimulation of the skin. The increase in the acidity of the gastric juice upon small injections of the amine is one of the more recent and delicate tests for this substance. According to Rachemann (1931) everything that is known about allergy tends to sub-

stantiate the ideas of Lewis.

In conclusion, the correspondence in susceptibility of the various species to histamine and to anaphylactic shock (Schmidt and Stahelein, 1929), is strongly suggestive of some interrelation between the two conditions. Those species which are very difficult to sensitize and shock are equally insusceptible to the histamine. Alteration of the susceptibility by diet or double adrenalectomy to one of the conditions also produces an identical change in the tolerance to the other. (Seegal and Khorazo, 1931, Wyman, 1928). Hence, with the similarity of the physiological reactions in mind, and considering the above correspondence in natural and altered resistance to histamine and anaphylactic shock, the parallelism between the two seems unusually complete.

METHODS

Colony management. The animals used in the following experiments were young, mature ($2\frac{1}{2}$ -6 months old), virgin rats, bred in this laboratory from a mixed stock of colored varieties of the albino rat M. norvegicus, originally obtained from the Bussey Institution of Harvard University. They received a varied and ample diet consisting of seed mixture, bread and milk, dog biscuit, and a balanced standard mash mixture. Fresh greens and cheese were supplied occasionally. Warmth, cleanliness, and dryness were maintained as well. Not more than three rats were housed in the same cage to avoid crowding

but isolation was found to be unnecessary even for the operated animals.

Operation. The adrenal glands were removed under ether anesthesia from two lateral apertures made in the dorsal muscles by blunt dissection just below the level of the last rib, according to a standard method (see Wyman, 1926) about one to two weeks prior to the course of sensitization. At the end of that period the animals had completely recovered from what little operative trauma had occurred, and had developed some degree of adrenal insufficiency.

To obtain growing, functional, autoplasmic cortical transplants, but no demonstrable chromaffin tissue, the glands immediately on removal were bisected and inserted into four superficial pockets in the M. obliquus abdominis externus, according to the method of Jaffe and Plavsky (1926). This operation was performed 6 to 12 weeks before sensitization in order to insure a sufficient amount of established transplanted tissue. Since the above method required assistance, one or two transplants were made in the dorsal muscles near the incision, in a few cases only.

Sensitization. The course of sensitization consisted of 6 daily intraperitoneal injections, or 3 on alternate days, of 0.5 cc. to 1.0 cc. of horse serum. The test dose was administered 9 to 14 days later. This procedure was found to be the most satisfactory by Parker and Parker and by Wyman. A

few preliminary experiments were carried out varying the number and amount of inoculations and the length of the incubation period, but no appreciable increase in sensitivity was produced. Accordingly the above plan of treatment was generally adhered to in all experiments for the study of anaphylactic shock to horse serum.

A small series of experiments were performed using crystallin egg albumin for comparison with the horse serum series. Passive sensitization was conferred by intraperitoneal injections of 1.0 cc. of a high titre anti-ovalbumin rabbit serum, 3 to 4 days prior to the shocking dose. The antigen and serum for these experiments were kindly furnished by Dr. S. B. Hooker of the Evans Memorial from a very limited supply.

In vitro experiments. The Schultz-Dale method was employed for the study of the reaction of the rat smooth muscle to histamine and in anaphylactic shock. The set-up of the apparatus was of the simple classroom type, except for a constant temperature bath which was available for most of the work. The muscle, attached by a fine thread to an L-shaped tube, which delivered oxygen from the tank through a small aperture at the elbow, was immersed in 200 cc. of Ringer-Locke solution at 37.5°C . The solution was contained in a large test tube of such dimensions that the muscle was adequately covered, and interference with the tissue from the stream of oxygen, and from the insertion of the thermometer and pipette for in-

jections, was avoided. The bath solution could be washed out and replenished by hydrostatic pressure through a two-way outlet at the base of the test-tube connecting with drain and with a reservoir kept at the same temperature. The upper end of the muscle was attached by silk to a light heart lever for recording the myograph on a moving smoked drum.

For convenience only one horn of the uterus was used, which was obtained as rapidly as possible after killing the animal by a blow on the head and exsanguination. The abdomen was opened by a median longitudinal incision. One horn was freed from the surrounding fat and broad ligament. Fine silk threads were tied directly beneath the ovary and around the base of the horn as close to the bifurcation as possible. In this way the full length obtained eliminated any source of error resulting from the use of different segments of the horn with different activity gradients. The lower end of the strip was tied to the glass rod, cut free from the vagina, and the horn with the ovary attached was placed immediately in the bath.

In vivo experiments. The usual method for obtaining a graphic record of the uterus has been to pin down a given segment to a bit of cork fastened in some way to the body wall, and to connect leads from various points of the attached sections to light heart levers for recording. Knaus flooded the abdomen with warm physiological solution. Another scheme met with in the literature was to submerge the entire body of the rat in Ringer's solution and proceed according to the Dale method.

The former method described presented many difficulties, namely, constant exposure of the organs, imperfect maintenance of the body temperature as well as that of the solution, insufficient excursion of the lever, interference of the intestines, and last but not least, pinning down of the segment and impermanence of the cork support.

After a few attempts to use or modify the above method, it was found more satisfactory to allow the horns of the uterus free play and to take off a lead from the thin strand of connective tissue found at the base of the bifurcation. In this way, waves of contraction passed down the horns under the thread. Recent studies have shown that the waves of contraction are initiated at the ovarian end and pass down the horns, the completeness of conduction and the initiation of extra contractions at other levels for the most part depend on the period of the oestrus cycle. The rat has been found to be rather more regular in this respect than other animals (Knaus and Clark, 1925; Blair, 1922; Harne, 1932). The rigidity of connections of the vagina and body of the uterus to surrounding structures did not allow any undue stretching or pulling of the organ outside of the body cavity. Although it was not absolutely necessary, a precaution was taken to keep the intestines from pressing on or moving upon the main portions of the uterine horns by slipping a small celluloid trough under them. This trough was shaped from a flat piece of the celluloid with the aid of hot water so as to have walls high enough to prevent the intestines from working over the edge, a smooth rolled lip

upon which the broad ligament rested without blocking the circulation or cutting the tissue, and a convex, triangular base which fitted securely over the dorsal wall of the abdomen and colon. After slipping the trough under the horns and tying the silk thread to the connective tissue at the bifurcation, the incision was sewed up except for a distance of about 1 cm. through which the thread passed up to the heart lever. The edges of this opening were held apart by a small wire retractor, so that the string was suspended absolutely free.

The operation was performed under urethane anesthesia as a routine, although a few control operations were done under ether. The femoral or external jugular vein was exposed for injections either by hyperdermic syringe or by cannula. The carotid artery was exposed, cannulated, and connected with a mercury manometer for the series in which blood pressure determinations were recorded (in regard to technique and apparatus, see Wyman and tum Suden, 1932) The pneumograph record was obtained by catching up a few hairs on the most movable part of the thorax in a heart-clip attached by a silk thread to another heart lever. Time was recorded in 5 second intervals by means of signal magnet actuated by an electric interrupter. All levers were arranged to write simultaneously on a smoked drum. Ten mgm. of heparin were injected intravenously to prevent clotting. (Hynson, Westcott, and Dunning Co.) Ergamine acid phosphate (Burroughs Wellcome & Co.) was used

for the histamine experiments.

RESULTS

Uterine reactions to histamine in vitro. A series of tests on the effect of various dilutions of histamine on the surviving uterus of the normal rat was chosen as a starting point for the present problem, not merely as a check on the past work but as a means for developing a standard technique for the bath method and for ascertaining the inherent characteristics of the material used. The first point is essential for such studies employing the usual simple set-up for bath experiments in order to maintain constant conditions in the matter of temperature, rate of oxygenation, lever tension, speed in obtaining the specimen and the least possible disturbance of the tissue on renewing the bath from one test to another. In the very elaborate apparatus which many recent workers have designed and used to insure the greatest possible accuracy, these physical conditions are controlled to a fine degree. Even so, the individual variation and irregularity of the normal activity appears as a definite characteristic of non-striated muscle.

In particular, the rat uterus has been said to be more variable and insensitive than the uteri from other species, namely the rabbit and guinea pig. Following the admonitions of Parker and Parker (1924) the first series of experiments was done on rats weighing from 50 to 80 gm. The authors claimed that uteri from rats of over 100 gm. were untrust-

THE UNIVERSITY OF CHICAGO

LETTER

TO THE PRESIDENT OF THE UNIVERSITY OF CHICAGO

FROM THE FACULTY OF THE UNIVERSITY OF CHICAGO

DEAR MR. PRESIDENT:

WE have the honor to acknowledge the receipt of your letter of the 10th inst.

and in reply to inform you that the same has been forwarded to the proper authorities.

We are, Sir, very respectfully, your obedient servant,

W. D. BOYD, Secretary of the Faculty.

Very truly yours,

W. D. BOYD, Secretary of the Faculty.

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worthy due to an increase in the frequency of erratic beats. They also observed that the limit of contractility and sensitivity of the rat smooth muscle was not as great as that of the guinea pig, which is readily recognized from the curves published in their paper. They also pointed out that in order to obtain the five recorded cases numerous preliminary tests were made, as so little work of this nature had been done on the rat.

The present work also involved a considerable number of preliminary experiments. Using Tyrodes solution and the uterine strips from young, immature rats, the rhythm and activity obtained in the more satisfactory cases was neither vigorous nor especially regular. Considerable variation in tonus was also noted in this group. However, in moderate dose histamine produced some degree of inhibition of the normal activity.

A few attempts were made to ascertain the effect of very dilute amounts of histamine on the rat uterus, since a few previous reports claimed that under these conditions the action of histamine was reversed. The tests were far from satisfactory because the responses were so slight that it was difficult to see sufficient departure from the normal curves to be sure of the action of the drug. Yet in three cases showing a slight, definite increase in tonus after histamine in concentrations of 1:1,000,000 to 1:5,000,000, there appeared to be little doubt that the rise could be

attributed to the effect of the drug.

Furthermore, the instances in which histamine in concentrations up to 1:500,000 produced no appreciable change in the record complicate the interpretation of the results. Three such cases are included in the series reported because they undoubtedly illustrated a natural unresponsiveness of the specimen. On examining the reactions of six other rat uteri to the same doses of histamine it was obvious that there was a varying degree of insensitivity to the drug. Unquestionable depression of the spontaneous activity was exhibited in all six experiments, but the response was not great in comparison with subsequent tests. Moreover, the effective dilutions varied from 1:1,000,000 to 1:300,000. The manifestations of depression such as a slight drop in tonus, a moderate decrease in rate for 2 to 8 minutes, a slight reduction in amplitude or, in the most frank examples, an arrest of all activity from 1 to 3 minutes, did not consistently correlate with the concentration. The cases B-14 and B-1, uterine strips from rats of approximately the same age and weight, showed a decided difference in reactivity. In the case of B-14, a dilution of 1:300,000 produced only a slight increase in the relaxation period between 2-3 consecutive contractions, whereas in the case of B-1 a dilution of 1:500,000 of the same solution tested a day or so later decreased the rate decidedly and lasted for 8 minutes. The most pronounced example of inhibition was obtained in a specimen from a rat weighing 100 gm. A dilution of

1:1,300,000 cut down the amplitude of the contractions immediately about 50 percent and the rate proportionately. During the next 40 minutes both rate and amplitude very gradually returned to normal.

It would appear from this series of experiments that the response to histamine was not as striking as others have reported it to be, except for one case. This seemed to be due to the fact that the specimen came from an older animal, so that the rhythm of the spontaneous activity was stronger and more regular. As the conditions in this case were the same as for the others, this factor of age seemed to exert a different influence than that reported by Parker and Parker. Another series was carried out on rats over 100 gms. in weight, substituting Ringer-Locke solution for Tyrode's. In these cases the relaxation produced by histamine was striking and instantaneous. In three cases the concentration of histamine was around 1:200,000, which produced a marked decrease in rate and amplitude and occasionally a very slight drop in tonus. In two instances the activity was completely arrested temporarily. In two other cases inhibition was produced in a concentration of 1:1,000,000. Later, in another series of rats of approximately the same weight, these results were repeated. The effective concentrations ran from 1:200,000 to 1:500,000, producing more or less complete cessation of activity for 3 to 6 minutes.

In general, it may be said that the above total of 24 cases showed that histamine caused depression of the normal

activity in dilutions up to 1:1,000,000 and that the degree of inhibition paralleled the dosage in some measure, but there seemed to be a considerable difference in the reactivity of different strips, especially in the higher dilutions. Very great dilutions may reverse the response but it is not constant or easily seen. Inhibition was more manifest in the older rats, where the increased activity and rhythmicity of the normal tracing afforded a good contrast to any change.

A few other features were noted in regard to the rat uterus. The effectiveness of repeated doses in most instances was less than ^{that of} the original dose, even when the muscle was washed out and placed in a fresh bath. Frequently two or three times the original concentration produced less or no response. The alteration of the H-ion concentration of the bath by histamine in concentrations up to 1:50,000 apparently had little influence on the muscle per se. Such variations as occurred (ph 8.5 to 7.7) had no noticeable effect on the normal activity when this change was produced by the addition of HCl. In three cases the effect of urethane as an anesthetic was tried, and it was found that there was no visible difference in the normal uterine activity or in its response to drugs. In agreement with the recent work on oestrus, the spontaneous contractions of the uterus were found to be more regular and best suited for comparative studies during the stages II and III, as there were fewer or no confusing submaximal, circular or composite

beats.

The response of the suspended uterine strip to histamine from a series of rats, 2 to 3 months old on the average and adrenalectomized about two weeks before, was the same as that obtained in the normal group. Slightly higher concentrations (1:200,000 and 1:400,000) in most instances were employed. In five cases very marked inhibition was seen --complete or almost complete arrest of all activity for a period of 5 to 20 minutes, according to the dose. In one case, a specimen from a very old rat showed complete inactivity for 11 minutes following the injection of histamine yielding a final concentration of 1:800,000. In six other cases the effect of histamine was not so marked, showing only an increased interval of relaxation between two consecutive contractions, or a decrease in amplitude and tonicity. Four cases exhibited no response to the drug. This may be attributed partly to difficulties in maintaining uniform conditions owing to the somewhat erratic rate of oxygenation and to the fact that the constant temperature bath was no longer available for this work. About 40% percent of the records for this group showed a decided irregularity in the rate of contraction. However, in tracings of the uterine movements from normal and transplanted rats made at this time, this feature was not so noticeable. Consequently one can not state whether or not the condition of adrenal insufficiency may have exerted some influence on the smooth muscle as has been found in conditions of vitamin deficiency (Hurwitz and

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Nichols, 1930), but in any case the effect of histamine was apparently unaltered.

The inhibitory effect of histamine was easily elicited from the isolated uterine strips obtained from rats which had functioning cortical material but no chromaffin tissue. The total number tested was only six, two of which were unsatisfactory due to inconstant temperature or rate of oxygenation. The depressing effect of histamine (1:400,000 to 1:1,000,000) was unmistakable, as the normal activity was fairly regular and vigorous. The amine produced more or less complete cessation in proportion to the dilution.

Some additional data were also obtained concerning the reactions of the rat uterus from normal and operated animals. It was found that adrenalin in very dilute amounts produced an immediate and complete relaxation lasting for a long period of time, in contrast to the effect of histamine. In a few instances, when histamine was added to the bath after the adrenalin, a slight resumption of the normal beating seemed to take place. Moderate to large doses of pituitrin produced contracture, and if histamine was injected during this phase a slight drop in tonus followed, but no striking counteraction of the pituitrin effect was noted. Following atropin, the response to histamine was not altered. The uterus, however, was considerably more sensitive to changes in the rate of oxygen flow and in temperature than to changes in pH and to drugs.

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← Inhibition or stimulation would occur after any change in the rate of the oxygen supply, especially if the rate increased. If the rate decreased, a transient increase in rate followed which gradually gave way to inactivity. A slight increase in temperature produced a gradual rise in tonus and rate, and in this latter condition histamine was less effective. If the tone was raised considerably, as when the temperature had inadvertently approached 40 C., fairly high concentrations of the amine were totally ineffective.

In regard to very dilute concentrations of histamine on the uterine strips from adrenalectomized and transplanted rats, it seemed rather unprofitable to pursue such a study with the apparatus available, judging from the results obtained in the groups of normal rats. However, in many cases higher dilutions were tested than the reported effective dose, with no response. It was found that as a general rule, in these two classes of rats as well as in the normal rats, the most certain minimal concentration of histamine eliciting a clear cut picture of inhibition was around 1:500,000 and occasionally 1:1,000,000.

Uterine reactions in anaphylactic shock in vitro. When normal horse serum, without preservative, was added to the physiological solution bathing a rhythmically beating strip of unsensitized rat uterine muscle, no change was produced in the tracing even with a dose as large as 1 cc. Occasionally one noted a slight increase in duration, amplitude or tone of a

contraction immediately following the injection. Old serum which may have developed some toxicity was more apt to exhibit this effect, but as even the freshly obtained serum acted in the same way on some muscle strips and not on others, it indicates that for some reason normal horse serum can exert a very slightly stimulating effect. Longcope (1922) found the same to be true. This fact did not interfere very decidedly in testing for anaphylaxis because the response of the uterus, when obtainable, was more striking and long lasting. Furthermore, after some time had elapsed and the bath had been renewed, a second test dose could be given, illustrating the contrast between the heightened response of the sensitized muscle and the decreased or total absence of a reaction in the partially or completely desensitized muscle. The tests on the unsensitized and desensitized muscle were controls not only for the effect of antigen, but also for the changes which occur in the bath on adding such a large quantity of serum, such as H-ion concentration, variations in salt concentration, and frothing from the lowering of the surface tension.

Fourteen normal rats, 2 to 3 months of age, were sensitized and tested as described before. A shocking dose of 1 cc. elicited in three cases a definite increase of activity and tone. Reinjection of the same dose later demonstrated almost a complete desensitization. In three other cases there was only a suggestion of the stimulating

contraction immediately following the injection. Old serum which may have developed some toxicity was more apt to exhibit this effect, but as even the freshly obtained serum acted in the same way on some muscle strips and not on others, it indicates that for some reason normal horse serum can exert a very slightly stimulating effect. Longcope (1922) found the same to be true. This fact did not interfere very decidedly in testing for anaphylaxis because the response of the uterus, when obtainable, was more striking and long lasting. Furthermore, after some time had elapsed and the bath had been renewed, a second test dose could be given, illustrating the contrast between the heightened response of the sensitized muscle and the depressed or total absence of a reaction in the partially or completely desensitized muscle. The tests on the unsensitized and desensitized muscle were controls not only for the effect of antigen but also for the changes which occur in the bath on adding such a large quantity of serum, such as K-ion concentration, variations in salt concentration, and frothing from the lowering of the surface tension.

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effect of 1 cc. of antigen, but a comparison with the control test and with the desensitization test indicated that the shocking dose was responsible for this response. The remaining eight cases were unquestionably negative, three of which had received only 0.01 cc. of antigen.

A similar series was carried out on 10 rats from which the adrenal glands had been removed 10 days or more before the course of sensitization was begun. As in the normal group the number of unmistakable positive cases was low, namely only two. In addition, there was a third of a suggestive character. The same difficulty with erratic rhythm and long periods of inactivity had to be contended with here as in the adrenalectomized group used for the histamine tests. In addition, the stimulating effect of horse serum in the control and desensitization tests was more prominent.

Only five cases comprised the series on the uterine response to anaphylaxis in rats with cortical transplants. About the same ratio of positive to negative cases was obtained. In two instances the shocking dose of 0.5 cc. of serum enhanced the number of submaximal contractions. This could also be interpreted as an increase in rate to such a degree that it incurred a decrease in amplitude. The remaining three cases were negative.

In conclusion, the results obtained on the three classes of rats by the bath method showed that in anaphylaxis the uterus is stimulated to contract, but that the response

effect of 1 cc. of antigen, but a comparison with the control test and with the desensitization test indicated that the shocking dose was responsible for this response. The remaining eight cases were unambiguously negative, three of which had received only 0.01 cc. of antigen.

A similar series was carried out on 10 rats from which the adrenal glands had been removed 10 days or more before the course of sensitization was begun. As in the normal group the number of unambiguously positive cases was low, namely only two. In addition, there was a third of a negative character. The same difficulty with certain types and long periods of inactivity had to be contended with here as in the adrenulectomized group used for the histamine tests. In addition, the stimulating effect of horse serum in the control and desensitization tests was more prominent.

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In conclusion, the results obtained on the three classes of rats by the bath method showed that in anaphylaxis the uterus is stimulated to contract, but that the response

is not marked or regularly obtained. Extremely large sensitizing doses and test doses of serum seemed to be required. There was apparently no difference in the response of the smooth muscle to shock or degree of sensitivity in the normal or operated rat.

Uterine reactions to histamine in vivo. The results of the bath experiments with the surviving uterus of normal and doubly adrenalectomized rats, and of rats with cortical transplants, showed no distinguishable differences in the reactions of these three types to histamine poisoning and to anaphylactic shock. As there is sufficient evidence proving that removal of the adrenal medulla increases the susceptibility to both these conditions in the intact animal, two possible explanations could account for the results obtained. The most likely one is that the change in susceptibility is not due to a difference in the reactivity of the smooth muscle but to an increased susceptibility of some other tissue or mechanism such as the reticulo-endothelial system. On the other hand, the above experiments are not conclusive proof that there is no change in the smooth muscle, since it is excised tissue; and it has been frequently suggested that this system should be studied in situ, where experimental conditions would be more nearly comparable to the normal physiological condition. One drawback is encountered, namely, that one cannot work with a constant, unchangeable, predictable medium. Yet there is sufficient reason to expect

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that the muscle in situ with intact blood and nerve supply, in a medium of tissue fluids at normal temperature, may show some points of variation from the excised, surviving muscle. With this in mind the responses of the uterus to histamine and in anaphylactic shock were studied in situ.

In seventeen normal rats the response of the uterus was observed upon the intravenous injection of varying doses of histamine according to the method previously described. The doses ran from 0.05 mgm. per 100 gm. body weight to 15.0 mgm.-100 gm., and as a rule the smaller doses were repeated. The average weight of the rats was 200 gms., this size being most convenient to handle. In most cases it was found that immediately upon injection there followed an almost imperceptible relaxation lasting about 30-45 seconds, in two cases injections of 10 to 15 mgm. produced this effect, which endured for 1 to 3 minutes. In most cases this manifestation of relaxation was due only to a slight drop in tone, hardly more than 2 to 3 mm. below the normal level. Accompanying this, in some cases as the only evidence, was a slight increase in the interval between two consecutive beats. There then followed a second phase in the activity of the uterus, which was a decided though slight increase in tone, rate and occasionally amplitude. This lasted from 6 to 30 minutes. Only two tests in this series showed no evidence of stimulation, and two other cases were merely suggestive. This second phase of increased activity was more definite than

that the muscles in situ with intact blood and nerve supply, in a medium of tissue fluids at normal temperature, may show some points of variation from the excited, surviving muscle. With this in mind the responses of the uterus to histamine and to epinephrine shock were studied in situ.

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the inhibitory phase and was quite marked when little or no spontaneous action could be elicited from the uterus previous to the injection, which was a frequent occurrence when working on normal rats.

In eight rats a record of the blood pressure also was taken. The dosage of histamine varied from 0.05 mgm. to 0.2 mgm.-100 gms. and produced the characteristic fall in blood pressure. From these simultaneous records it was noted that the inhibitory phase closely corresponded in time of onset and duration with the precipitous decline in blood pressure, and that the period of increased activity commenced either at the moment when the blood pressure reached the bottom or had just started to recover. The stimulation of the uterine activity was not dependent upon the level of the blood pressure; that is it was not necessary for the pressure to regain its former level or even to approach it, for in those cases where a large dose of histamine was given, the level of the blood pressure remained low, regaining less than 10 mm. Hg; and yet this phase was seen. If a lethal dose was given the situation changed. The systemic effects produced considerable interference with the tracings, especially the respiratory spasms. Moreover, as the blood pressure gradually declined the uterine activity also gradually faded out until death. With such large doses one could not obtain any information about the actual effect of the amine on the muscle.

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These results indicate that with small injections of histamine from which the animal recovered, the effect on the uterus was very slight. It seems that the relaxation is but transitory and almost insignificant, and cannot be truly attributed to the effect of the drug but rather to the sudden and profound fall in blood pressure, since this correspondence in time was so close. It seems unlikely that the histamine could reach the muscle in effective concentrations so nearly instantaneously. The phase of increased activity is also debatable, but appears to be more likely due to a small concentration of the drug reaching the uterus. Furthermore, a few experiments on the effect of vagus stimulation, amyl nitrite, and very dilute adrenalin, all of which produced a sudden fall in blood pressure, also showed a transient inhibition of the normal uterine activity corresponding with the period of the decline in pressure. In these instances the stimulation phase was not visible.

Intravenous injections of histamine in doses from 0.1 to 1.0 mgm. per 100 gms. body weight produced the same effects on the uterus of doubly adrenalectomized rats as it did on the normal rats. The series consisted of 12 cases, of which two cases were untrustworthy because the response was so faint. In the other 10 cases the period of relaxation was as transitory and slight as in the normals. The decrease in tone was somewhat more visible due to adjustment of the lever. The period of increased activity was not so marked

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tamine from which the subject recovered, the effect on the
arteries was very slight. It seems that the relaxation is not
transitory and almost instantaneous, and cannot be directly attrib-
uted to the effect of the drug but rather to the action and
prolonged fall in blood pressure, since this correspondence in
time was not observed. It seems unlikely that the histamine could
reach the muscle in sufficient concentration to produce such a
response. The phase of increased activity is also delayed,
but appears to be more likely due to a small concentration of
the drug reaching the artery. For example, a few experiments
on the effect of venous stimulation, very slight, and very
slight increases, all of which produced a similar fall in blood
pressure, also showed a transient inhibition of the arterial
activity corresponding with the action of the histamine
in general. It seems therefore the action time factor was not
variable.

Intermittent injections of histamine in doses from 0.1 to
1.0 mgm. per 100 c.c. body weight produced the same effects
on the uterus of deeply anesthetized rats as it did on
the normal rats. The action consisted of 12 cases, of which
two cases were unresponsive because the response was so
small. In the other 10 cases the period of relaxation was
transitory and slight as in the normal. The duration
in some was somewhat more variable for a statement of the
effect. The period of increased activity was not so marked

and occurred in only 7 out of the 10 rats. The remaining three cases showed the initial inhibition with the fall in blood pressure, and the failure of the second phase to appear could be accounted for by the fact that all three succumbed directly to a repeated dose of histamine of 0.4 mgm. and 0.1 mgm. per 100 gms. while even the first injections of 0.05 mgm and 0.1 mgm. - 100 gm. caused profound systemic effects with no recovery. As mentioned before, uterine activity gradually ceased when the animal became more or less moribund. Rats without cortical and medullary tissue were not very satisfactory for experiments of this nature, as they were often sickly at the start and did not withstand the operative and experimental procedures for any length of time.

In addition to the above group there was one case in which a drop of histamine (conc. 1:1000) was applied to the uterus in situ. In this instance definite inhibition followed.

The same type of experiment was carried out on a series of 10 rats with functioning cortical tissue but no medullary tissue. In contrast to the doubly adrenalectomized group these animals were in vigorous health and withstood the operative procedures very well. In only two cases where the doses of histamine were 2 mgm. per 100 gms. body weight, were the readings of the tracings impossible due to systemic collapse. In the other eight cases the two phases of relaxation and increased activity were the same as found in the normal group. In a group in which no blood pressure

and occurred in only 3 out of the 10 rats. The remaining three cases showed the initial inhibition with the fall in blood pressure, and the failure of the second phase to appear could be accounted for by the fact that all three animals died at a repeated dose of histamine of 0.5 mg. and 0.1 mg. per 100 gms. while even the first injection of 0.05 mg. and 0.1 mg. - 100 gms. caused profound systemic effects with no recovery. As mentioned before, uterine activity gradually ceased when the animal became more or less moribund. Data obtained, however, and preliminary results were not very satisfactory for experiments of this nature, as they were often cloudy at the start and did not resemble the qualitative and experimental procedures for any length of time.

In addition to the above group there was one group in which a drop of histamine (0.1 mg.) was applied to the animal in situ. In this instance the inhibition followed the same type of experiment was carried out on a series of 10 rats with transverse cervical incision but no preliminary incision. In contrast to the results obtained in the first group, these animals were in vigorous health and exhibited the operative procedures very well. In only two cases where the dose of histamine was 1 mg. per 100 gms. body weight, were the readings of the transverse incision low. The results obtained in the first group were the same as found in the normal group. In a group in which no blood pressure

was recorded the average dose ran from 0.2 mgm. to 2.0 mgm. per 100 gms. body weight, but in the group with simultaneous blood pressure determinations the dosage did not exceed 0.5 mgm. per 100 gm. because all the previous experiments indicated that these low doses were more satisfactory and uncomplicated by respiration, gradual decline of the blood pressure or general failure of the rat. In these rats it was noted that normal activity of the uterus was consistently greater than that obtained in the normal group. It seems possible that this circumstance is due to the fact that in normal animals the adrenals may be stimulated to liberate adrenin, which has been shown to inhibit the activity of the uterus very effectively. Knaus (1925) has reported that 1/500 th of the adrenin stored by the gland is sufficient to eliminate the uterine contraction. In the present work it was found that a direct application of adrenalin to the oozing skin incision at the neck completely inactivated all uterine contraction for over $\frac{1}{2}$ hour.

Response of the uterus to anaphylactic shock in vivo. For the study of anaphylactic shock the in-situ method used was the same as that in the histamine experiments. The animals received from 3 to 5 daily injections of 0.5 to 1.0 cc. of horse serum, and from 10 to 15 days following the last sensitizing dose the animals were tested for shock by intravenous injections of 0.5 to 1.0 cc. of the serum. The operation

was recorded the average dose ran from 0.2 mgm. to 2.0 mgm. per 100 gms. body weight, but in the group with simultaneous blood pressure determinations the dosage did not exceed 0.5 mgm. per 100 gm. because all the previous experiments indicated that these low doses were more satisfactory and unobscured by respiration, gradual decline of the blood pressure or general failure of the rat. In these rats it was noted that normal activity of the uterus was considerably greater than that obtained in the normal group. It seems possible that this circumstance is due to the fact that in normal animals the adrenals may be stimulated to liberate adrenalin, which has been shown to inhibit the activity of the uterus very effectively. Brown (1935) has reported that 1:500 of the adrenalin stored by the gland is sufficient to eliminate the uterine contraction. In the present work it was found that a direct application of adrenalin to the posing skin resulted in the back completely involuntarily all uterine contraction for over a hour.

Exposure of the uterus to anaphylactic shock in vivo. For the study of anaphylactic shock the in-vivo method used was the same as that in the histamine experiments. The animals received from 5 to 8 daily injections of 0.5 to 1.0 cc. of horse serum, and from 10 to 15 days following the last sensitization some the animals were tested for shock by intravenous injections of 0.5 to 1.0 cc. of the serum. The operation

necessitated using fully grown rats, so that their weights ran from 150 to 200 gms. In the earlier group of experiments records of the blood pressure and respiration were not taken, but later, when the apparatus was so arranged and adjusted that these records could be made on the same drum by use of the long paper, electric kymograph, a second group of experiments was carried on supplementing these data along with the uterine response in normal, doubly adrenalectomized and transplanted rats.

The results of the experiments on 10 sensitized normal rats without blood pressure determinations afforded only one case in which the evidence derived from the reaction of the uterus was distinctly positive. In this instance the rate, tone and amplitude of the contractions were increased about one minute after the injection. No other symptoms were noted, and the animal survived. In five other rats the respiration became slightly labored a few minutes later and then became normal. This in itself was not considered sufficient proof of an anaphylactic condition although later it was found that respiratory difficulties were generally an accompaniment of the decline in blood pressure.

It was unfortunate that shock experiments on the normal sensitized rat with blood pressure determinations were started at a time when circumstances arose so that only three rats were available. In these three experiments only two of the uterine records showed any response to shock. In $3\frac{1}{2}$ and 7

necessitated using fully grown rats, so that their weights ran from 150 to 200 gm. In the earlier group of experiments records of the blood pressure and respiration were not taken, but later, when the apparatus was so arranged and adjusted that these records could be made on the same drum by use of the long paper, electric kymograph, a second group of experiments was carried on supplementing these data along with the uterine response in normal, doubly adrenomedullarized and transplanted rats.

The results of the experiments on 15 normal rats without blood pressure determinations afforded only one case in which the evidence derived from the reaction of the uterus was distinctly positive. In this instance the rate, force and amplitude of the contractions were increased about one minute after the injection. No other symptoms were noted, and the animal survived. In five other rats the respiration became slightly labored a few minutes later and then became normal. This in itself was not considered sufficient proof of an anaphylactic condition although later it was found that respiratory difficulties were generally an accompaniment of the reaction in blood pressure.

It was unfortunate that blood experiments on the normal rats failed to give any blood pressure determinations were started at a time when circumstances arose so that only three rats were available. In these three experiments only two of the uterine records showed any response to shock. In 3g and 3h

minutes after the test dose the tone of the uterus increased slightly. In all three cases, however, the blood pressure fell and the uterine response appeared just afterward. No changes occurred in respiration and no pathological findings were observed in the gastro-intestinal tract at biopsy. The average initial blood pressure level was 111.3 mm. Hg, which dropped to 66.6 mm. Hg during the course of 10 minutes. The normal level was regained in about one-half hour. All survived. Taking the group as a whole, there were only 4 mildly positive cases out of 13.

Eight out of 10 sensitized doubly adrenalectomized rats in this series, in which no blood pressure records were made, suffered from fairly profound anaphylactic shock. In six of the eight the effects were fatal 14 to 120 minutes after the injection. The uterine response was not striking or comparable to the degree of shock seen. In two cases which were considered as positive, due to the respiratory disturbance along with congestion of the gut, the uterus remained unaffected. One of these rats died in 26 minutes. In two other cases, one fatal, the gastro-intestinal symptoms were not seen, but the uterus responded by a slight increase in rate and number of submaximal contractions. All 10 rats developed some dyspnea several minutes after the shocking dose, but as in the normal group this could not be considered in itself as sufficiently reliable to classify them all as positive.

minutes after the test began the tone of the uterus increased slightly. In all three cases, however, the blood pressure fell and the uterine response appeared just afterward. No changes occurred in respiration and no pathological findings were observed in the gastro-intestinal tract at autopsy. The average initial blood pressure level was 111.5 mm. Hg, which dropped to 66.8 mm. Hg during the course of 10 minutes. The normal level was regained in about one-half hour. All survivors. Taking the group as a whole, there were only a slightly positive cases out of 15.

Eight out of 10 animals died shortly after the test. The rate in this series, in which no blood pressure records were made, suffered from fairly profound anaphylactic shock. In six of the eight the effects were fatal in 15 minutes after the injection. The uterine response was not striking or comparable to the series of shock seen. In two cases which were considered as positive, due to the respiratory disturbance along with congestion of the gut, the uterus remained unaffected. One of these rats died in 15 minutes. In two other cases, one fatal, the gastro-intestinal symptoms were not seen, but the uterus responded by a slight increase in rate and number of contractions. All 10 rats developed some degree of general anesthesia after the shock, but as in the normal group this could not be considered as itself a sufficiently reliable or clinically than all as positive.

In the series of doubly adrenalectomized rats in which blood pressure changes were observed, the response of the uterus was found again to be less noticeable and less constant than the other effects of shock. The fall in blood pressure and the gastro-intestinal congestion occurred in all the seven positive, but not in the one negative case. The respiratory spasms were almost a constant finding and in fact seem to be associated in most instances with the low level of the blood pressure, as it did not appear until after the fall had taken place. Six of the group died in from 32 to 131 minutes. In the remaining positive case the blood pressure returned to normal after 40 minutes. Another fairly constant feature was, that following the injection of antigen there was a latent period of 3 to 4 minutes, the fall in blood pressure and the increased activity of the uterus occurring at about the same time. The drop in pressure occupied several minutes, averaging about 10 minutes. It was a rather marked change, although by no means the precipitous decline such as that brought about by a sudden injection of histamine. Having reached a low level (average 48.0 mm Hg) further change came about very gradually. In contrast to this condition in shock are the two instances where the rat was not sensitive to the antigen, one because sensitization failed and one in which desensitization developed when the rat in question recovered. In both cases injections of horse serum of 1 cc. raised the blood pressure and maintained it for a period of many minutes, even longer

In the series of double cannulizations in which blood pressure changes were observed, the responses of the system were found again to be less noticeable and less constant than the other effects of shock. The fall in blood pressure and the extra-intestinal congestion occurred in all the series positive, but not in the one negative case. The respiratory changes were almost a constant finding and in fact seem to be associated in most instances with the fall of the blood pressure, as it did not appear until after the fall had taken place. Six of the group died in from 15 to 151 minutes. In the remaining positive case the blood pressure returned to normal after 45 minutes. Another fairly constant feature was that following the injection of antivenom there was a latent period of 5 to 6 minutes, the fall in blood pressure and the increased activity of the system occurring at about the same time. The drop in pressure occupied several minutes, averaging about 10 minutes. It was a rather marked change, falling to 70 mm Hg and the respiratory distress such as that observed about 15 minutes after injection of antivenom. Having reached a low level (average 55.5 mm Hg) further change was about very gradually. In contrast to this reaction in which the two instruments were the red was not sensitive to the antivenom, one became sensitized failed and one in which sensitization developed when the red in question recovered. In both cases injection of horse serum of 1 cc. raised the blood pressure and maintained it for a period of many minutes, even longer

than one-half hour. The return to normal was very slow and steady. In control rats, which will be discussed later, horse serum injections did not produce the syndrome described here.

In the series of sensitized rats with transplanted cortical tissue in which no blood pressure determinations were made, six of the eleven developed definite anaphylaxis, three dying within 45 minutes after the injections. As two cases were tested on the 5th day of incubation, no reaction followed; and these served as additional controls. In three other cases the only indication was the labored breathing in 6 to 10 minutes. These again were not included in the positive group. In the six positive cases the reaction of the uterus was very slight, and in only three truly significant. However, in the whole group of positives the respiratory symptoms and the gastrointestinal findings substantiated the faint uterine indication.

In addition, nine rats were tested for shock and the blood pressure changes determined. Seven were positive, one was negative and one was doubtful. Only one died. Again the uterine response was faint. A slight elevation of the tone and a possible increase in rate with some additional submaximal contractions occurred about the time or slightly after the blood pressure started to fall. This was also followed by dyspnoea. After 30 minutes to one hour three animals showed a partial recovery of the blood pressure and in four cases no appreciable change had taken place. On autopsy all but one exhibited congestion of the gastro-intestinal tract.

then one-half hour. The response to control was very slow and steady. In control rats, which will be discussed later, control serum injections did not produce the apnoeic described here. In the series of sensitized rats with transplanted cortical tissue in which no blood pressure determinations were made, six of the eleven developed definite apnoeic, three dying within 45 minutes after the injections. In the series were tested on the 5th day of incubation, no reaction followed; and these served as additional controls. In three other cases the only indication was the labored breathing in a 10 minute period. These again were not included in the positive group. In the six positive cases the reaction of the serum was very slight, but in only three truly significant. However, in the whole group of positives the respiratory apnoea and the gastro-intestinal findings substantiated the fact of the positive reaction. In addition, also rats were tested for shock and the blood pressure changes determined. Seven were positive, one was negative and one was doubtful. Only one died. Again the reaction response was slight. A slight elevation of the blood and a possible increase in rate with some additional endothelial contractions occurred about the time or slightly after the blood pressure started to fall. This was also followed by apnoea. After 30 minutes to one hour there was a partial recovery of the blood pressure and in four cases no appreciable change had taken place. On autopsy all but one exhibited congestion of the gastro-intestinal tract.

Upon comparing the difference in the effect of the test dose of horse serum in the sensitized normal, doubly adrenalectomized and transplanted rats it was easily seen that the latter two classes exhibited more profound systemic effects, and as a group contained a larger percentage of positive and fatal cases. The percentage of positive uterine reactions may have been slightly higher than in the normals, but the response was on the whole no different in character from that exhibited by the normals. In all instances the reaction of the uterus in situ was scarcely distinct or constant enough to be chosen as the one unquestionable criterion of anaphylaxis.

It should also be noted that in the three groups in which blood pressure determinations were made, the most constant and delicate indication of shock in the rat was the fall in blood pressure. The manifestations of increased permeability of the intestinal mucosa and exudation of blood into the lumen of the gut was seen only in rats where shock was fatal or where recovery was prolonged or failing to occur. Respiratory distress seemed to be in some way dependent upon the fall in pressure or the low level attained. In the matter of the increased number of deaths among the doubly adrenalectomized groups, the facts that these rats has a low blood pressure at the start of the experiments and also were not in the best of health as compared with the other groups, undoubtedly contributed to the early death. The average initial blood pressure for the adrenalectomized series was

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 dose of horse serum in the sensitized animal, clearly animals
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 response was on the whole no different in character from
 that exhibited by the normal. In all instances the reaction
 of the serum in rats was generally distinct or constant enough
 to be chosen as the one representative criterion of sensitivity.
 It should also be noted that in the three groups in
 which blood pressure determinations were made, the rats were
 kept and handled in the same manner as the rat was the
 rat in blood pressure. The maintenance of blood
 pressure of the individual animal and oxidation of blood
 into the lungs of the rat was seen only in rats where shock
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 pressure at the start of the experiment and also with the
 in the best of health as compared with the other groups,
 undoubtedly contributed to the early death. The average
 initial blood pressure for the sensitized rats was

75.1 mm Hg, while that of the transplants was 104.0 mm Hg and the normals 111.3 mm. Hg. In addition to this it should be recalled that although only 4 deaths were reported for the entire group of 20 transplants, yet in the series in which blood pressure determinations were made several of the animals did not attain complete recovery within the same period of time required by the normals. It is undoubtedly true (although Wyman reported in 1929 that the doubly adrenalectomized and transplanted rats were equally susceptible to shock) that in these experiments the extensive operation complicates the outcome to some degree, but in no way invalidates the conclusion that both types of rats are more susceptible to shock than normal rats. The response of the uterus is apparently the same in all three types.

Anaphylaxis in the rat passively sensitized to ovalbumin. For the study of anaphylaxis foreign serum, especially horse serum, has been selected by many of the investigators because it is easily secured, inexpensive, and non-toxic for the rat. From an immunological standpoint it is far from ideal because of its complexity and the uncertainty of the reacting constituents. Another commonly used antigen is egg albumin. However, Novy and de Kruif (1917) claimed that this was primarily toxic for the rat and could even cause death. Because of the difficulty in sensitizing normal rats it was thought that by using a purified antigen, i.e. egg albumin, a more dependable picture

10.1 at 50, while that of the transplants was 104.8 at 50 and the normal 111.5 at 50. In addition to this it should be recalled that although only 4 deaths were reported for the entire group of 25 transplants, yet in the series in which blood pressure determinations were made several of the subjects did not attain complete recovery within the same period of time required by the normal. It is undoubtedly true (although Ryan reported in 1937 that the death rate was low) and transplanted rats were usually susceptible to shock) that in these experiments the extensive operation complicated the outcome to some degree, but in no way invalidated the conclusion that both types of rats are more susceptible to shock than normal rats. The response of the system is apparently the same in all three types.

Asphyxiation and the rat passively submitted to asphyxiation. For the study of asphyxiation foreign workers, especially those who have been selected by many of the investigators because it is easily secured, insensitive, and non-lethal for the rat, from an immunological standpoint it is far from ideal because of its complexity and the uncertainty of the resulting conclusions. Another commonly used method is eye asphyxiation. However, May and de Gault (1917) claimed that this was primarily fatal for the rat and could even cause death. Because of the difficulty in maintaining normal rats it was thought that by using a purified antigen, i.e. eye albumin, a more dependable picture

of anaphylaxis could be obtained and would serve as a check on the results from the horse serum series if the toxicity factor proved to be less troublesome than reported. It was also hoped that passive sensitization to a single antigenic protein could enhance the response to anaphylaxis.

A small quantity of purified ovalbumin, and a high titre anti-ovalbumin rabbit serum for sensitization, both prepared in the Evans Memorial, were kindly supplied by Dr. S. B. Hooker. Normal rats, doubly adrenalectomized rats, and rats with cortical transplants were passively sensitized by intraperitoneal injections of 0.25 to 1.0 cc of the anti-serum 3 to 4 days before the test dose of purified antigen was given by vein. Blood pressure, respiration and the uterine response in situ were recorded as before.

After the injection of 0.4 to 0.6 cc of 5 per cent antigen into 3 normal rats, not a single sign of shock was seen although at the moment of injection the blood pressure fell precipitously and just as rapidly returned to normal. This latter was the toxic reaction as it occurred also in the non-sensitized control rats.

In two passively sensitized, adrenalectomized rats the test dose of 0.4 cc. of 5 per cent ovalbumin produced fatal shock in one and a moderate degree in the second case. In the fatal case there was a slow, steady fall in the blood pressure from 48 mm. Hg to 35 mm. Hg., starting five minutes after the injection. After 20 minutes the low level was reached and

It is suggested that the results should be interpreted with caution as a result of the fact that the results from the two series are not directly comparable. It was found that the results from the two series are not directly comparable. It was found that the results from the two series are not directly comparable.

A small quantity of purified ovalbumin, and a high titre anti-ovalbumin rabbit serum for sensitization, both prepared in the Evans Memorial, were kindly supplied by Dr. E. W. Hooker. Rabbit sera, locally seroprecipitated sera, and sera with control responses were passively sensitized by intraperitoneal injections of 0.25 to 1.0 cc of the anti-serum. To 4 days before the test dose of purified ovalbumin was given by vein. Blood pressure, respiration and the skin response in skin were recorded as before.

After the injection of 0.5 to 0.6 cc of 2 per cent ovalbumin into a normal rabbit, a small skin wheal was seen 15 minutes at the point of injection and blood pressure fell precipitously and heart rate rapidly returned to normal. This latter was the toxic reaction as is pointed out in the non-sensitized rabbit.

In two passively sensitized, adrenalectomized rats the first dose of 0.5 cc, at 2 per cent ovalbumin produced fatal shock in 15 and 20 minutes. In the second dose, in the third dose, there was a skin wheal, slightly fall in the blood pressure from 110 to 90 mm. Hg., respiration five minutes after the injection. After 20 minutes the low level was recorded and

maintained for some time, but after one hour it dropped to zero. There was no apparent response of the uterus. The respiration became irregular and labored as the blood pressure reached the low level, and then improved until shortly before death. On autopsy, congestion of the intestines was seen.

In the second case, although the rat recovered, the effect of shock was even more typical. Within 20 minutes the blood pressure had fallen from 80 mm Hg. to 58 mm. Hg., starting 9 minutes after the injection, and was accompanied by slight dyspnoea. Within 25 minutes the pressure had returned to normal. Reinjection of the same amount of antigen produced the immediate transient dip in pressure, but the normal level was maintained thereafter, illustrating well the phenomenon of desensitization. The uterine response was very slight, but clear. The tone and rate increased 12 minutes after the injection and lasted for about 10 minutes. After the normal rhythm was regained no further change took place even after the second injection. In the one control experiment for adrenalectomized rats, the sudden injection of ovalbumin produced this same fall as described here. No changes in respiration, uterine activity or condition of the intestinal tract were seen.

The intravenous injection of 0.5 to 1.0 cc of 1 per cent ovalbumin caused shock in five passively sensitized rats with cortical transplants. Three cases were fatal. On the average the blood pressure fell about 30 to 40 mm Hg during a period of 30 to 40 minutes, starting 5 to 10 minutes after injection. The

maintained for some time, but after one hour it began to
rise. There was no apparent response of the uterus. The res-
piration became irregular and labored as the blood pressure
reached the low level, and then improved until shortly before
death. On autopsy, congestion of the intestines was seen.
In the second case, although the rat recovered, the blood
pressure was even more typical. Within 10 minutes the blood
pressure had fallen from 80 mm Hg. to 35 mm Hg., starting 5
minutes after the injection, and was accompanied by slight
dyspnea. Within 15 minutes the pressure had returned to
normal. Rejection of the same amount of undiluted pyrogen
the immediate transient dip in pressure, but the normal level
was maintained thereafter. Administration with the pyrogenous
of bacteriolysin. The uterine response was very slight, but
clear. The temperature increased 1/2 degree after the in-
jection and lasted for about 10 minutes. After the normal
rhythm was resumed no further change took place even after the
second injection. In the one control experiment for chemical-
induced fever, the rabbit injected at midnight produced this
same fall as described here. No changes in respiration, gastric
activity or position of the intestinal tract were seen.
The intravenous injection of 0.5 to 1.0 cc of 1 per cent
evulsin caused shock in five partially sensitized rats with
cortical symptoms. These cases were fatal. On the average
the blood pressure fell about 50 to 60 mm Hg during a period of
30 to 45 minutes, starting 5 to 10 minutes after injection. The

respiratory, gastro-intestinal and uterine reactions were not particularly marked or consistently present. Only two cases showed a satisfactory change in the activity of the uterus. In case E-12, which terminated fatally, a slight rise in tonus occurred. In E-14, which recovered, there was a definite though slight increase in the rate from 11 to 13 beats per 15 minutes. One control experiment was done which showed that the injection of ovalbumin per se was not responsible for the above finding.

The results from this group of experiments indicate that the onset of anaphylaxis to egg albumin was not as sudden or as rapid in development as that obtained to horse serum. A more extensive series would be necessary in order to determine finally this point as well as the possibility that a lighter degree of shock developed after passive sensitization to a purified^{ied} antigen. However, there is sufficient evidence from these few experiments to show that the egg albumin anaphylactic shock did not differ in any feature except intensity from that obtained in the horse serum groups, and that the comparative susceptibility of the three types of rats was just as evident.

Controls. In the work on the isolated surviving muscle the introduction of drugs into the bath was controlled by the addition of normal saline or Ringer-Locke solution. This showed that the method used for injection in no way disturbed

respiratory, gastro-intestinal and circulatory reactions were not particularly marked or consistently present. Only two cases showed a satisfactory change in the activity of the organs. In case E-12, which terminated fatally, a slight rise in temperature occurred. In E-14, which recovered, there was a definite though slight increase in the rate from 11 to 15 beats per 15 minutes. One control experiment was done which showed that the injection of ovalbumin per se was not responsible for the above findings.

The results from this group of experiments indicate that the onset of anaphylaxis to egg albumin was not as sudden or as rapid in development as that obtained in horse serum. More extensive series would be necessary in order to determine finally this point as well as the possibility that a slight degree of shock developed after passive sensitization to a built-in antigen. However, there is sufficient evidence from these experiments to show that the egg albumin anaphylaxis shock did not differ in any feature except intensity from that obtained in the horse serum groups, and that the comparative susceptibility of the three types of sera was just as evident.

Conclusions. In the work on the isolated surviving mouse the introduction of virus into the body was controlled by the addition of normal saline or higher-lactose solution. This showed that the method used for infection in the key findings

the muscle. In experiments employing drugs such as pituitrin, adrenalin, etc., the response of the uterine muscle served as controls for the reactivity.

In the bath experiments on anaphylaxis the effect of horse serum was tested on the non-sensitized uterus. The rats were sensitized in groups of 3 to 6 at a time, and one or two rats from the same litter or operated group was set aside as controls for the particular lot of serum used for the inoculation of the given group. The reaction of the sensitized uterus was always compared with its own control because, as was mentioned before, the non-sensitized uterine strip might react slightly to normal horse serum. In addition to this, the reinjection of the same amount of serum after shock had taken place and the bath had been refreshed afforded the best control, as one obtained here a direct comparison of its effect on the same muscle and under the same conditions. The addition of 0.5 to 1.0 cc of the antigen to the bath brought about a change in the H-ion concentration and in the surface tension of the medium surrounding the tissue, which in themselves might lead one to expect some change in the automatic beating or tone. However, the frothing and the lowering of the pH did not produce any visible reaction on the part of the tissue. As a check on this insensitivity to pH changes, a few experiments were carried out on the effect of lowering the pH by addition of dilute hydrochloric acid. No effect could be seen as the pH was adjusted to between 8.2 and 7.5,

the muscle. In experiments involving human skin as a substrate, etc., the response of the skin muscle served as a basis for the results.

In the first experiments on amphibians the effect of force exerted was tested on the non-muscular tissue. The results were summarized in groups of 3 to 5 at a time, and one of the tests from the same litter or operated group was not used as a control for the particular lot of animals used for the experiment of the given group. The reaction of the sensitized animals was always compared with the own control response, as was mentioned before, and non-sensitized animals might react slightly to normal saline serum. In addition to the points mentioned above, the same amount of serum after exposure to each glass and the bath had been returned elicited the same control, as one obtained with a 5% solution of the effect on the same animal and under the same conditions. The addition of 0.5 to 1.5 cc of the antigen to the bath brought about a change in the W-bath concentration and in the response of the animal surrounding the tissue, which in these cases was found to be about the same in the antigenic testing of tests. However, the findings and the lowering of the pH did not produce any visible reaction on the part of the tissue. As a check on this immaturity in the changes, the experiments were carried out on the effect of lowering the pH by addition of dilute hydrochloric acid. No effect could be seen as the pH was adjusted to between 6.5 and 7.5.

the range which included any possible changes induced by histamine or horse serum.

The matter of adequate controls for the experiments *in situ* offered some difficulty from the facts that anesthesia was required and that the anticoagulant, heparin, was necessary in order to obtain records of the blood pressure. The intravenous injection of 10 mgm. of heparin was chosen in preference to sodium citrate in the manometer system because in former work it was found that when citrate entered the circulatory system during a fall in blood pressure, undesirable and confusing systemic disturbances ensued. The only objection to the use of heparin is that many workers have maintained that it has a protective action in anaphylaxis, (Keyes & Strauser, 1926; Zunz & Van Geertyden, Bernard, 1921). However, this is denied by others, (Hill & Martin, 1932). After surveying the entire series, including all three types of rats, it seemed that it had little or no influence on the course of shock and if any it was a constant one for three types so that the comparative susceptibility remained the same. As for a possible influence on the uterine reactions, the series in which no blood pressure determination were done showed that this organ was not noticeably altered in its response by the presence of the anticoagulant.

The customary procedure in bath experiments has been to kill the animal by a blow on the head. The main reason for this has been to obtain the smooth muscle in as nearly a

normal state as possible by avoiding the introduction of extraneous factors such as anesthetics. This course was followed in the routine bath work, but a few experiments were carried out in the bath on uteri from rats which had been inactivated by 0.15 mg - 100 gm body weight of urethane. Urethane was selected because it supposedly induces rapid and profound narcosis with little change in the respiration and the circulation. It in no way altered the normal activity or the reaction of the uterine muscle to the substances under investigation. It therefore seemed likely that the use of this anesthetic for the shock experiments in situ would not confuse the issue. Two experiments were run under ether anesthesia. It is generally accepted that ether augments the reaction to histamine. Since intravenous injections showed so very little effect on the uterus, and that more in the nature of a reversal than that obtained in the bath, it seemed plausible that under ether the inhibitory phase previously described might be more pronounced. However, this did not reveal itself. It was concluded that the lack of response of the uterus to the intravenous injections of histamine was not due to the anesthetic, but rather to the failure of the drug to reach the muscle in sufficient concentration to effect inhibition through the blood stream. This fact was further substantiated by the experiment in which a drop of histamine applied directly to the exposed surface of the intact uterus produced inhibition.

Some normal saline controls were also run. Previously

normal state as possible by avoiding the introduction of extraneous factors such as anesthesia. This course was followed in the routine bath work, but a few experiments were carried out in the bath on short rats which had been anesthetized by 0.15 mg - 100 mg body weight of urethane. Urethane was selected because it supposedly induces rapid and profound narcosis with little change in the respiration and the circulation. It in no way altered the normal activity of the respiration of the animals exposed to the hypoxia under investigation. It therefore seemed likely that the use of this anesthetic for the whole experiment in this work would not confuse the issue. The experiments with the urethane-anesthetized rats are generally accepted as being suggestive of the reaction to hypoxia. Since intravenous injections showed a very little effect on the respiration and that there was no evidence of a reversal when that condition in the bath, it seemed probable that under other conditions phase previously described might be more pronounced. However, this did not reveal itself. It was concluded that the lack of response of the animals to the intravenous injections of hypoxia was not due to the anesthesia, but rather to the failure of the drug to reach the glands in sufficient concentration to effect liberation through the blood stream. This fact was further substantiated by the experiment in which a drop of hypoxine applied directly to the exposed surface of the intact animal produced inhibition.

Some normal saline controls were also run. Previously

in other problems, the effect of injections of normal saline on the blood pressure had been found to be innocuous (Wyman and Sudent, 1932a, 1932b). These tests were designed to find out if the addition to the circulation of fluid in amounts customarily employed contributed any influence on the reaction of the uterus. Apparently the injections of 1 cc. did not produce sufficient increase in the blood volume to bring about a significant elevation of the blood pressure to effect thereby the uterine activity.

The experiments on anaphylaxis, with and without blood pressures, were controlled by the usual injections of non-sensitized rats with horse serum from the same lot which had been used for the course of sensitization of the other rats in the particular group to be tested. Desensitization tests were carried out on all rats, where possible, as the most adequate and demonstrable proof of anaphylaxis.

Several experiments were directed to the effect of the sudden lowering of the blood pressure by amyl nitrite and faradic stimulation of the vagus on the normal uterine activity. It was found that at the moment of onset of the decline of the pressure there was a very slight indication of relaxation. This lasted, as well as could be judged, as long as the fall, and then immediately returned to normal. Stimulation of the vagus was found not to be entirely satisfactory, the reaction being by far too rapid and fleeting because of 'vagus escape.' The amyl nitrite inhibition also allowed for some criticism,

in other conditions, the effect of injections of normal saline
 of the blood pressure had been found to be insignificant (Wynne and
 his colleagues, 1934, 1935). These results were referred to show
 out of the relation to the circulation of fluid in certain
 necessarily employed conditions any influence on the reaction
 of the system. Apparently the injection of 1 cc. did not
 produce sufficient increase in the blood volume to bring about
 a significant elevation of the blood pressure to affect thereby
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The experiments on anaphylaxis, with and without blood
 pressure, were conducted by the usual injections of hyp-
 sensitized sera into horses drawn from the same lot which had
 been used for the purpose of sensitization of the other two
 in the previous group to be tested. Desensitization tests
 were carried out on all rats, where possible, as the most
 adequate and systematic proof of anaphylaxis.
 Several experiments were directed to the effect of the
 sudden lowering of the blood pressure by nylalazine and
 the effect of the vagus on the normal uterine activity.
 It was found that at the moment of onset of the decline of the
 pressure there was a very slight inhibition of relaxation.
 This lasted, as well as could be judged, as long as the fall,
 and then immediately returned to normal. Stimulation of the
 vagus was found not to be entirely satisfactory; the reaction
 being by the too rapid and intense pressure of vagus escape.
 The nylalazine inhibition was allowed for more or less

as the action of the drug is presumably on the contractile substance, relaxing smooth muscle in general without reference to the innervation.

Pituitrin and adrenalin injections were also given. This showed that the characteristic action of these drugs was readily elicited in situ. In particular, it was noted that the inhibitory effect of adrenalin was as marked in situ as in the bath, as seen from the previous reference to the effectiveness of a local application to the skin incision around the neck. This seemed to afford a possible explanation of why the normal rat uterus so often appeared less active than in the medulliadrenalectomized cases in situ; it is in accord with the evidence presented by Cannon (1931) that in normal animals an operative disturbance causes a considerably exaggerated medulliadrenal secretion.

DISCUSSION

The usual effect of histamine on the isolated uterus of the normal rat is one of relaxation. This is in agreement with the findings of Guggenheim 1912, Fuhner 1913, Cow 1914, Ogata 1921, Longcope 1922, Voegtlin and Dyer 1925, etc. The same reaction was obtained both in the normal and in the operated rat muscle strips regardless of previous sensitization or shock. Voegtline and Dyer (1925) claimed that a concentration of 1:3,000,000 elicited this response quite regularly and that relaxation was obtained in even higher

dilutions. On the other hand, Kelloway (1930) found that he could only procure constant relaxation in dilutions not greater than 1:1,000,000. Longcope (1922), Cow (1919), and Abel (1919) employed higher concentrations to demonstrate this action. In the bath experiments reported here, it was found that although relaxation occurred at times in dilutions up to 1:2,000,000, the effect of histamine was likely to be obscured because the fluctuation in the normal spontaneous contractions were of the same magnitude and kind. Moreover, the individual variation in sensitivity was more apparent in the higher dilutions. As pointed out by Abel and Kubota and by Cow, the rat uterine muscle is not so sensitive to histamine as that from other species, even though the effect is in the opposite direction. The guinea pig uterus contracts definitely in a concentration of 1:10,000,000. Abel demonstrated an unquestionable reversal in the action of the drug on the mouse uterus in dilutions of 1:2,000,000 and 1:1,000,000; but in the rat, only a dilution of one to "several" millions produces reversal, which was in no way as definite as that seen in the mouse. In 1918 Adler also observed this action on the part of the mouse uterine muscle. There are very few reports on the rat uterus in regard to histamine reversal except a mention by Feldberg and Schilf, of work by Katz and the sporadic, uncertain instances referred to by Best, Dudley, Dale and Thorpe in their work with extracts of histamine from normal tissues. However, Best and McHenry (1931) do not seem to challenge the validity

of these reports but state that the fact needs further substantiation.

When the present problem was begun it was intended to investigate the response of the surviving uterus to very minute doses of histamine with this possibility in mind, but the vagueness and inconstancy of the results discouraged further work until all probable extraneous factors, attributable to technique and apparatus, could be eliminated. The three instances in which reversal was observed suggested that it might have been possible, under ideal conditions, to obtain an adequate and reliable confirmation of the stimulating effect of small amounts of histamine on the rat uterus.

Relatively little work has been done on the effect of drugs on the uterus in situ. In the rat, Knaus and Clark (1925) and Gunn and Gunn (1914) studied the effects of certain drugs and salts in vivo, and found that the response was the same as for the excised muscle. No reference to similar work on the effect of histamine has been found. Reynolds and his associates (1930) developed a method for the study of the rabbit uterus in situ without using anesthesia. In regard to histamine they found that the systemic effects of the drug interfered with the tracing of the uterine response and that with doses small enough to eliminate this difficulty no assured response could be detected. The same situation was encountered in the present work on the rat. In contradistinction to the results obtained in the bath experiments, the

of these reports but state that the fact needs further sub-
stantiation.

When the present problem was begun it was intended to
investigate the response of the surviving leaves to very
slight doses of distance with this possibility in mind, but
the vagueness and inconsistency of the results discouraged
further work until all probable extraneous factors, attributable
to technique and apparatus, could be eliminated. The three
instances in which reversal was observed suggested that it
might have been possible, under ideal conditions, to obtain
an adequate and reliable indication of the self-light effect
of small amounts of distance on the eye itself.

Relatively little work has been done on the effect of
distance on the observer in situ. In the past, however, Ogle
(1935) and Ogle and Goss (1934) studied the effects of certain
doses and levels in vivo, and found that the responses were
more or less similar. He concluded that similar work
on the effect of distance was well founded. Hough and his
associates (1930) developed a method for the study of the
visual system in situ without using anesthetics. In regard to
distance they found that the systemic effects of the drug
introduced with the tracing of the observer response and that
with doses small enough to eliminate this difficulty no
measured response could be detected. The same reaction was
observed in the present work on the eye. In conclusion,
attention to the results obtained in the later experiments, the

pure inhibitory action of histamine could not be clearly demonstrated in situ by intravenous injections, but could be elicited by direct application of a fairly strong solution to the exposed surface of the uterus in vivo. In most cases a slight stimulating action was more pronounced than the immediate, transient relaxation. In normal rats where the possible liberation of adrenin into the circulation almost inactivated the automatic rhythm, the phase of increased tone and activity was very prominent. The transient depression could be interpreted as a secondary effect because of the speed of onset, momentary duration, and the magnitude of the reaction. Barbour and Rapaport (1922) demonstrated on the uterus of the dog in situ that activity was abolished under conditions establishing a deficient circulation to the organ, such as hemorrhage and injections of hypertonic salt solution. The results of the experiments in which amyl nitrite and vagal stimulation were employed suggested this possibility. When adrenalin or pituitrin was injected, the uterine response was not so instantaneous as this first apparent effect of depression, but showed that a slight latent period was required before the drug reached the muscle. As a general rule, the Schultz-Dale method has been found to be a fairly reliable, simplified, physiological means to reproduce in vitro, qualitatively if not always quantitatively, the reactions of non-striated muscle to various substances. The evidence cited here, though meagre, definitely signifies that the difference in the

The inhibitory action of nicotine could not be clearly demonstrated in this type of experiment, but could be suggested by direct application of a fairly strong solution to the exposed surface of the uterus in vivo. In most cases a slight stimulating action was more pronounced than the immediate transient relaxation. In normal cases where the possible liberation of adrenalin into the circulation almost immediately stimulates the system, the phase of increased tone and activity was very prominent. The transient depression could be interpreted as a secondary effect because at the onset of onset, however, and the magnitude of the reaction. Evidently, as reported (1932) demonstrated on the uterus of the dog in situ that activity was abolished under conditions entailing a deficient stimulation to the system, such as hemorrhage and in-
 formation of hypotonic and solution. The results of the experiments in which any nitric acid and vaginal stimulation were employed suggested this possibility. When stimulation of the uterus was initiated, the uterine response was not as instantaneous as this first apparent effect of depression, but showed that a slight latent period was required before the first response was recorded. As a general rule, the Schmitt-Dale method has been found to be a fairly reliable, simplified physiological means to reproduce in vitro, qualitatively if not always quantitatively, the reactions of non-stimulated uterus to various substances. The various cited facts, though scarce, definitely suggest that the difference in the

response in vivo and in vitro may be primarily a quantitative one.

In view of the possibility that excised muscle is stimulated by very minute quantities of histamine, it is very probable that the slightly increased rate and tone obtained in situ is a manifestation of this point. It is not at all unlikely that when the drug is administered via the circulation, the numerical potency of its action on the muscle is far less than the calculated value, and even below that of any concentration tested in the bath. Knaus and Clark (1925), in regard to the effect of adrenalin on the rat uterus, demonstrated that "the concentration required to produce an effect in situ is from 40 to 100 times that which acts upon the isolated muscle". For their study in situ the drug was given by vein. The results obtained here with histamine may offer a parallel case. The usual dose of 0.1 and 0.2 mgm per 100 gm. would bring the concentration of the drug in the blood up to 1:100,000 or 1:200,000, figuring on the basis of 15 to 20 cc total blood volume for the rat of 200 gms. body weight (Went and Drinker, 1929, Wyman, 1930). One would expect that the high theoretical concentration of the amine in the blood would act in the same direction as in the bath or on direct application. Considering the complex chemical constitution of the blood, it is unlikely that the calculated potency of the drug obtains. In the first place it is known that histamine is readily oxidized in the body. Best and McHenry (1929, 1930) have proven that kidney, intestinal and

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one.

In view of the possibility that excited states in atoms

formed by very minute quantities of substance, it is very probable

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when the drug is administered via the circulation, the measured

potency of its action on the muscle is far less than the cal-

culated value, and even below that of any concentration tested

in the bath. (Krebs and Clark (1933). In regard to the action

of adrenaline on the rat uterus, Rosenkrantz (1934) has demon-

strated that the action on uterus in vitro is due to the

100 times that which acts upon the isolated muscle. For their

study in vivo the drug was given by vein. The results obtained

here with adrenaline may offer a partial answer. The amount of

0.1 and 0.2 mg per 100 ml. would give the concentration

of the drug in the blood up to 1:100,000 or 1:200,000, depending

on the basis of 15 to 20 cc total blood volume for the rat of

200 gm. body weight (Krebs and Briggs, 1934; Watanabe, 1934).

One would expect that the high theoretical concentration of the

adrenaline in the blood would act in the same direction as in the

bath on an intact organism. Considering the possible molecular

regulation of the blood, it is unlikely that the calculated

potency of the drug obtained in the first place is in error

that adrenaline is really diluted in the body. (Krebs and

McNulty (1934, 1935) have shown that activity, intestinal and

blood tissue, and particularly lung tissue, contain a specific enzyme for histamine destruction. Percival and Scott (1931) have also demonstrated that in the presence of serum histamine is inactivated. Using the rat uterus as a test object, they found that a dilution of 1:1,000,000 was inhibitory, while a dilution of 1:10,000 was required in the presence of serum. Salant and Parkins (1932) have reported a similar situation. The inhibitory dose of ergotamine (1:50,000) for the isolated cat intestine was rendered ineffective in the presence of 20 to 25 per cent blood. Furthermore, under such conditions, with dilutions of 1:100,000 and 1:200,000, the action was frequently reversed. In the cat and rabbit they also observed depression of the intestinal movements *ex situ*, but *in situ* the opposite occurred which they likewise inferred to be a quantitative matter.

Other factors may contribute some influence on the response of the uterus *in situ*, but there is little or no pertinent evidence concerning such a relationship to the action of histamine. Dale showed that the systemic reaction to histamine was considerably aggravated under ether narcosis. It is generally recognized that drug reactions may be enhanced or depressed by the presence of the serum proteins, colloids and lipoids, and other foreign agents such as ether. (Storm van Leeuwen, and Szent Gyorgyi 1921, 1922, 1925). The paper by Salant and Parkins covered an extensive investigation on the influence of ions on the action of ergotamine on the intestine of cats,

black islands, and particularly lung tissue, contain a specific enzyme for histamine destruction. Ferkovskii and Scott (1951) have also demonstrated that in the presence of serum histamine is inactivated. Using the rat uterus as a test object, they found that a dilution of 1:1,000,000 was inhibitory, while a dilution of 1:10,000 was revealed in the presence of serum. Salant and Ferkovskii (1952) have reported a similar situation. The inhibitory dose of epinephrine (1:50,000) for the isolated rat intestine was rendered ineffective in the presence of 10 to 25 per cent blood. Furthermore, under such conditions, with dilutions of 1:100,000 and 1:500,000, the action was quantitatively reversed. In the rat and rabbit they also observed depression of the intestinal movements *ex vivo*, but in this the opposite occurred which they likewise referred to as a quantitative action.

Other factors may contribute some influence on the response of the uterus in vitro, but there is little or no pertinent evidence concerning such a relationship to the action of epinephrine. This shows that the systemic reaction to histamine was considerably exaggerated under other conditions. It is generally recognized that this reaction may be enhanced or depressed by the presence of the various proteins, colloids and lipoids, and other foreign agents such as ether. (Scott van Loon, and Scott (1951, 1952, 1953). The paper by Salant and Ferkovskii is an extensive investigation on the influence of fat on the action of epinephrine on the intestine of rats.

rabbits, and rats. They were able to produce a definite modification, and even reversal, of the ergotamine inhibition by increasing the absolute concentration of calcium, especially in conjunction with a decided elevation of the H-ion concentration. But one could question the physiological import of such modifications, since the required alteration of the calcium content of the nutrient solution and the decrease in pH were beyond the limits normally found in the body. It was interesting to note that they also found that the rat intestinal smooth muscle was relatively less sensitive to the higher dilution of the drug than that of the rabbit or cat, and was only slightly affected, if at all, by pH changes until well beyond neutrality. In regard to the influence of cations on the rat uterine muscle, Knaus and Clark showed that potassium increased the rate of conduction, and that calcium produced a definite loss of tone. Kennedy (1925) estimated that doubling the content of the calcium chloride in the bath medium rapidly eliminated the tone of the suspended muscle; and found that the Dale modification of Ringer's solution, in which the percentages of calcium and potassium are respectively 0.024 per cent and 0.042 per cent, was more satisfactory. It has been previously mentioned that the normal spontaneous activity in these experiments improved on using Ringer-Locke solution instead of Tyrode's solution, in which the percentage of calcium and potassium are the same. Heller and Holtz (1932) also found that spontaneous contractions of the immature guinea pig uterus was abolished

...and water. They were able to produce a definite ...
...and even reversal, of the ...
...the absolute concentration of ...
...in conjunction with a ...
...But one could question the physiological import of ...
...such modifications, since the ...
...also content of the ...
...were beyond the limits ...
...interesting to note that they also found that the ...
...that smooth muscle was relatively ...
...higher dilation of the ...
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...definite loss of ...
...the content of the ...
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...relation, in which the ...
...the ...
...contractions of the ...

in Tyrode's, whereas in the modified Ringer-Locke solution the normal rhythm was maintained. The absolute content of calcium can be a point of serious concern in experiments on excised tissue where the choice of the nutrient medium may create a difference of 100 per cent in calcium as well as modify the Ca:K ratio. In situ however, in the rat, it seems that the fluctuation of serum calcium is never as great as this normally and seldom approaches such a change even in pathological conditions. According to the figures given in Donaldson's book on the rat, the average serum calcium is 11-12 mgm per 100 cc blood. According to Kramer and Howland (1922) the normal value seemed to be about 9-10.5 mgm per cent. Under very drastic dietary deficiencies the lowest level obtained by them was 4.5 mgm per cent; while Schelling (1932) was able to produce hypercalcemia (19.7 mgm per cent) with very marked pathological symptoms by continued overdosage with vitamin D. In histamine shock and in immunological reactions the changes found in the diffusible calcium and potassium were not comparable to the above figures. La Barre (1926), unlike Kuschinsky (1929), found no change in the calcium level after injections of histamine. Zimmermann (1931) also claimed that potassium was increased about 26 per cent. According to Best and McHenry (1931), these changes may be attributed in some extent to the increased concentration of the blood. With regard to the influence of the cations on the action of histamine upon the excised muscle, it was found that dilutions up to 1:1,000,000 inhibited the

In Tyndale's, whereas in the activated Ringer-Rosen solution the
hazardous reaction was maintained. The absolute content of calcium
can be a point of serious concern in experiments on calcium
levels where the choice of the nutrient medium may greatly
influence the rate of calcium in solution as well as modify the
response. In this respect, it is clear that the
reaction of calcium solution is never as great as this normally
and calcium supplements such a change even in physiological con-
ditions. According to the figures given in Donaldson's book
on the rat, the average serum calcium is 11-12 mg per 100 cc
blood. According to Jones and Jones (1951) the normal
value seemed to be about 9-10.5 mg per cent. Under very drastic
dietary deficiencies the lowest level observed (7 mg per cent) was
less than 5 mg per cent; while Gosselin (1952) was able to produce hyper-
calcemia (17.7 mg per cent) with very small parathyroid
system by continuous overdosage with vitamin D. In his experi-
ment and in physiological reactions the changes found in the
alkaline solution and parathyroid were not comparable to the
above figures. In fact (1950), while Knecht (1954) found
no change in the calcium level after injection of vitamin D,
Hirschman (1951) also obtained rapid parathyroid and increased
about 50 per cent. According to Best and Roberts (1951),
these changes may be attributed in some extent to the increase
concentration of the blood. With regard to the influence of
the calcium on the action of vitamin upon the existing animal,
it was found that calcium up to 1:1,000,000 inhibited the

uterus in Tyrode's as well as in Ringer-Locke solution. Abel obtained reversal in Ringer-Locke's while the few instances of reversal seen here occurred in Tyrode's solution. Although the normal automatic activity may have been influenced by the bath medium, the response to the drug was not noticeably altered by this factor. Concerning the sensitivity of the uterus in situ to changes in the cation values, which are even less than that which occurred ex situ, there is no other available source of information except the behavior of the excised strip.

Since potassium and calcium directly affect the irritability of nerve, a few investigators suggest that changes in the ion relationship in serum are associated with an alteration in the nervous mechanism and with the stimulation of non-striated muscle in anaphylactic shock. (Green and Bonham, 1924, Berthelson and Murdick, 1931, Zimmermann, 1931, Stoland, Sherwood, and Woodbury, 1931). Since the work was carried out on other species than the rat, this statement includes histamine shock as well.

This point of view would necessitate consideration of the site of action of histamine and other autonomic drugs. Ogata (1921) confirmed Gunn and Gunn (1924) and Langley and Anderson (1895) in that the innervation of the rat uterus is through the hypogastric nerve and is predominantly inhibitory. In contradiction to Elliot, he believed that the enhanced reaction to drugs after nerve degeneration in his experiments was not due to an increased sensitivity of the nerve endings,

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Since potassium and sodium directly affect the irrita-
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ducted impulse in myofibrillar shock. (Green and Brown,
1954; Bernstein and Katchik, 1951; Zimmerman, 1951; Stohr,
Sherwood, and Woodbury, 1951). Since the work was carried out
on other species than the rat, this statement includes his-
tory known as well.

This point of view would necessitate consideration of
the site of action of histamine and other autonomic drugs.
Gale (1951) confirmed Gale and Dunn (1951) and Langley and
Anderson (1935) in that the innervation of the rat uterus is
through the sympathetic nerve and is predominantly inhibitory.
In contrast to this, he believed that the enhanced
reaction to drugs after nerve denervation in his experiments
was not due to an increased sensitivity of the nerve endings.

but rather to the altered tone of the horn in which the nerves were sectioned for comparison with the normal intact horn. He obtained the same results using barium chloride, which acts directly on the muscle fibre. In the opinion of Knaus and Clark (1925), who investigated the drug and salt action on the rat uterus, the behavior of the smooth muscle is dependent upon the change in the rate of conduction, which they were inclined to believe was partly or entirely a function of the muscle fibre. There is no conclusive proof that histamine acts on nerve endings or even on the hypothetical receptive mechanisms, favored by Sollman (1922) as the seat of action for most autonomic poisons. The consensus of opinion is that histamine acts directly on muscle (Best and McHenry, 1931, Cushney, 1928). The following summary of the situation was obtained from the monograph on histamine by Feldberg and Schilf (1930):

A relationship of histamine to the autonomic innervation of smooth muscle is not established. It stimulates where the sympathetic is inhibitory. Again, in other cases, the effect is the same as sympathetic stimulation. The so-called vegetative poisons, as atropin and nicotine, have in most cases no influence on the course of the histamine action. Further, histamine is effective on the foetal smooth muscle before innervation has been established.

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were recorded for comparison with the normal latent period. We
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nerve endings or even on the hypothetical sensitive membrane,
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faint smooth muscle before innervation has been
established.

Therefore it does not seem likely that the difference in response of the uterus in vivo and in vitro can be attributed to variations in the salt balance or to the intact nervous mechanism. Nevertheless the evidence so far obtained points to some quantitative difference in the effective concentration reaching the tissue.

The response of the rat uterus to anaphylactic shock is one of contraction, but it is an extremely weak reaction which is not elicited constantly either in vivo or in vitro. Kelloway (1930) found that the uterus responded positively with more regularity after passive sensitization. Although my results on the actively and passively sensitized series do not warrant any exact comparison because of the limited number of cases and the different antigens used, nevertheless, there was no indication that passive sensitization enhanced the uterine reaction. No conspicuous difference in the type of response was observed in the intact or excised uterus. The same was true for the type of response obtained in normal rats, in adrenalectomized rats, or in rats with cortical transplants. However, a higher percentage of positive uterine reactions was obtained in vivo than in vitro. In the bath experiments the percentage of positive cases ran about 40 per cent, and no significant variation in this average between the three types of rats was noted. In situ, the percentage of positive reactions was higher in the operated animals. Yet it was found that only 60 to 70 per cent of the cases exhibiting shock, as

Therefore it does not seem likely that the difference in response of the neuron in vivo and in vitro can be attributed to variations in the salt balance or to the intact nervous mechanism. Nevertheless the evidence so far obtained points to some quantitative difference in the effective concentration reaching the tissue.

The response of the rat retina to anaphrodisia is one of contrast, but it is an extremely weak reaction which is not elicited consistently either in vivo or in vitro. Kellaway (1950) found that the retina responded positively with more regularity after massive sensitization. Although my results on the retinal and possibly sensitized retina do not warrant any exact comparison because of the limited number of cases and the slight anaphrodisia used, nevertheless, there is no indication that positive sensitization enhanced the response. No consistent difference in the type of response was observed in the intact or excised retina. The same was true for the type of response obtained in normal rats, in anaphrodisia rats, or in rats with cortical transplants. However, a higher percentage of positive response was obtained in vivo than in vitro. In the bath experiments the percentage of positive cases ran about 40 per cent, and no significant variation in this average between the three types of rats was noted. In vivo, the percentage of positive responses was higher in the operated animals. Yet it was found that only 50 to 70 per cent of the cases exhibited anaphro-

judged by other symptoms, was accompanied by stimulation of the uterus. This value for the three types of rats was surprisingly consistent. The increased susceptibility of the rat without adrenal medulla to anaphylaxis was quite obvious. When the blood pressure was recorded as a source of information the positive reactions in the three groups of rats closely approached 100 per cent. This particular manifestation, nevertheless, better illustrated the difference in sensitivity. In the operated shocked rats recovery frequently failed to set in or was prolonged and only partial, in contrast to the normal shocked rats where recovery was rapid and complete. There was no instance of lethal shock in the 15 normal rats tested for anaphylaxis.

The increased susceptibility of the rat to anaphylaxis after the loss of the adrenal medulla appears to be due to the irrecoverable circulatory collapse, from the fact that the response of the excised tissue did not show this same difference in sensitivity. On the other hand, in situ, the higher percentage of positive cases in rats suffering from the loss of the adrenal medulla was accompanied by a proportionate rise in positive uterine reactions. This finding would be interpreted by many as indicating that a higher degree of sensitization was possible in the operated animal since all tissues seem to be involved in the process. According to the theory of the cellular site of the anaphylactic reaction, the immediate

induced by other symptoms, was accompanied by stimulation of the
arteries. This value for the three types of rats was substantially
constant. The increased susceptibility of the rat to anaphylaxis
after removal of the adrenal medulla was quite obvious. When the
blood pressure was recorded as a source of information the
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proached 100 per cent. This percentage manifestation, never-
theless, better illustrated the difference in sensitivity.
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ation was possible in the operated animal than in the normal.
According to the theory, according to the theory,
of the cellular site of the anaphylactic reaction, the immediate

response is brought about by an interaction of antigen in or on the sensitized cell, and not as a secondary effect from a disturbance elsewhere in the body. If these conditions held absolutely, the response of the uterus should have been an entirely independent reaction, and if such were the case one would not anticipate a difference in the sensitivity of the organ in vivo and in vitro. If a higher degree of sensitization had been procured in the operated rats, the excised uterus should have reflected this difference, not only in an increased number of positive reactions but also in the magnitude of the reaction. For this reason one is led to believe that the increased number exhibiting shock in these two groups is governed to some degree by the loss of a protective mechanism against shock. Fairly conclusive evidence for this view has been produced by Flashman (1925) and Wyman (1929) who found that in the rat the increased susceptibility to anaphylaxis was quite independent of the process of sensitization; for in rats sensitized before operation and receiving the test dose of serum after removal of the adrenal glands, the number and severity of the positive cases increased. Secondly, the present series, which included blood pressure determinations, also illustrated the fact that the initial, major fall in pressure was practically as great in the operated as in the unoperated cases; that is, the extent of the fall in mm. correlated with the initial height of the blood pressure rather than with the low level finally attained.

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This again seems to imply that an inadequate defense mechanism is a reasonable explanation for the visible manifestations of shock in adrenalectomized rats.

Such an interpretation of the results of this study are obviously in harmony with the idea that the modification of the resistance of the rat to anaphylactic shock parallels the similar situation in regard to drugs and poisons. In all these conditions such a change has been attributed to a defective defense mechanism (Wyman, 1929; Crivellari, 1926; Scott, 1924; Lewis, 1925). As Wyman (1929) showed, this inadequacy is apparently due, at least in the case of histamine, to the loss of the medulla and not the cortex of the adrenal glands. The close resemblance in pathological and outward manifestations between histamine and anaphylactic shock strongly suggests that histamine liberation plays an important part in the anaphylactic symptoms of the rat.

The reactions of the dog and the rat to both these conditions are very much alike and certain immunological peculiarities are common to both species as well (Opie 1924). Manwaring has demonstrated in the dog that the liberation or formation of a toxic depressor substance is for the major part responsible for the clinical syndrome. The work of Dragstedt and Gebauer-Feulnegg also indicated the formation of histamine in the dog and very recently they have been able to produce even stronger evidence of the nature of this toxic agent obtained from the thoracic lymph or vena caval blood (Nov. 1932). This

substance, though not as yet isolated in pure crystalline form, has been found to give many specific reactions identical with that of histamine: namely, lowering of the blood pressure in the etherized, atropinized cat; contraction of the isolated guinea pig smooth muscle; relaxation of the mouse uterine muscle; positive wheal formation in the human skin. Chemically, it is basic, thermolabile, inactivated by diazotized sulphanilic acid, and is a crystalloid of relatively small molecular size, which is not specific immunologically. This evidence is exceedingly strong for the support of the theory that histamine plays an active part in the manifestation of anaphylactic shock, but it is only strictly applicable to the case of the dog. Objections could be raised on the ground of species variations if one claimed that the evidence was sufficient for the rat. However, it can be inferred from the similarity of the reaction of the dog and of the rat to anaphylaxis and histamine shock, in which the major symptoms in both species is one of increased capillary permeability and circulatory collapse, that somewhat the same course of events may take place. In the matter of species variation in these two conditions, the dog is more susceptible than the rat. However, after double adrenalectomy in the rat one finds that the resistance to both is equally lowered. Another peculiarity of the rat, which is found in no other species except the mouse, is the inhibitory influence of histamine on the isolated uterus. This fact has been emphasized as an important differ-

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...muscle; positive chronotropic action in the human heart. Finally,
...only, it is basic, thermolabile, inactivated by alkalies
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...isolated substance, which is not specific immunologically. This
...evidence is exceedingly strong for the aspect of the theory
...that histamine plays an active part in the mechanism of
...anaphylactic shock, for it is only actively capable of the
...role of the histamine. Questions could be raised as to the amount
...of histamine released in anaphylactic shock, but the evidence was not
...sufficient to answer them. However, it can be inferred from the
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...circulatory collapse. It is evident that the same course of events
...may take place. In the case of a severe reaction in these
...two animals, the dog is more susceptible than the cat.
...However, there is a marked difference in the way the two
...the reaction to histamine is usually observed. Another possibility
...of the way, which is found in the guinea pig and the
...mouse, is the inhibition of histamine in the isolated
...intestine. This has been suggested as an important differ-

ential point between anaphylactic shock and histamine shock. As far as one can ascertain from the literature this reaction has been studied only with the excised muscle, and the majority of investigators have used fairly high concentrations of the drug. Only Voegtlin and Dyer obtained inhibition with comparatively small dosages, and no others have been able to absolutely confirm them in these high dilutions. Few attempts if any, except that of Katze, have been directed to an inquiry into the reversal of the histamine action on the rat uterus obtained by Abel. In this problem the reaction of the rat uterus to intravenous injections of histamine was observed, and it was found that inhibition such as observed in the bath, did not occur. The muscle appeared relatively little affected by the drug, although a mild delayed stimulation was most likely due to the amine; the immediate previous, transient, cessation of the normal activity could not be definitely attributed to a direct action of the drug on the muscle. The striking feature of the uterine reaction, however, was that it closely resembled that obtained in anaphylactic shock in situ. Moreover this resemblance extended as well to the systemic disturbances, with one difference, namely that the decline in blood pressure was considerably slower in onset and in duration of the fall than that obtained by an intravenous injection of the drug. Obviously this point demonstrates a very definite time factor required for the production of the depressor substance. Practically coincident with the

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man. However this resemblance extended as well as the
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and in duration of the fall than that obtained by an intra-
venous injection of the drug. Obviously this point resem-
bles a very definite time factor required for the production
of the depressor response. Practically coincident with the

fall of blood pressure was the response of the uterus.

Reynolds, Weinstein et al. (1931) observed this same delay in the uterine response to anaphylaxis in the unanaesthetized rabbit.

In view of what has been obtained here, it appears that the results tend to support the idea that the powerful inhibition produced by histamine in the rat uterus suspended in the bath is not a true picture of its effect in situ. Therefore the response of the excised uterus to histamine has less significance as a differential point in the discussion of the relation of histamine to anaphylaxis. The results are more in accord with Dale's opinion, which is as follows:

"We may picture the anaphylactic shock, therefore, as the result of cellular injury, due to the intracellular reaction of the antigen with an aggregating antibody. Whether this is general, or localised in a particular organ, histamine will be released, and its effects will be prominent in the resulting reaction, imposing a general resemblance to the syndrome produced by histamine itself, on the symptoms seen in each species. The cell injury however, is not limited to the degree required to produce a release of histamine and involves other and more direct results. Such a conception is in accordance with all the facts as yet available, and it has the advantage of rendering intelligible, not only the striking resemblance between symptoms

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however, is not limited to the organs reported to

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and more direct results. Such a suggestion is in

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and it has the advantage of explaining the results

not only the striking resemblance between systemic

of the anaphylactic reaction and those produced by injecting histamine, but also the various and equally significant points of difference between the two syndromes."

SUMMARY


1. Histamine depresses in the rat the spontaneous activity of the uterus in vitro, in dilutions of at least up to 1:1,000,000.
2. Intravenous injections of histamine, from 0.05 mgm. to 5.0 mgm. per 100 gms. body weight, do not produce inhibition in situ similar to that seen with the excised muscle, but appears to cause a mild stimulation of tone and rate.
3. The response of the rat uterus to anaphylactic shock in vivo and in vitro is one of mildly increased activity.
4. The predominant reaction in anaphylactic shock and in histamine shock in the rat is the fall in blood pressure.
5. Double adrenalectomy or medulli-adrenalectomy decreases the resistance of the rat to both types of shock, however, without appreciably altering the reactivity of the uterine muscle.
6. The similarity between the systemic reactions, as well as between the uterine reactions in situ, of the rat to histamine and in anaphylactic shock indicates that the evidence against the liberation of histamine in anaphylaxis, based on the difference in the response of the excised uterus to both these conditions, is not conclusive.

of the anaphylactic reaction and those produced by intracutaneous histamine, but also the various and equally significant points of difference between the two syndromes."

SUMMARY

1. Histamine depresses in the rat the spontaneous activity of the uterus *in vitro*, in dilutions of at least 1:100,000.
2. Intravenous injections of histamine, from 0.05 mgm. to 5.0 mgm. per 100 gm. body weight, do not produce marked effects on the uterus *in vitro* or on the uterine blood flow, but produce a mild depression of the uterine blood flow.
3. The response of the rat uterus to anaphylactic shock *in vivo* and *in vitro* is one of mildly increased activity.
4. The post-anaphylactic reaction in anaphylactic shock *in vivo* is similar to that in the rat in that the uterine blood flow is depressed.
5. Double anaphylaxis or multiple anaphylaxis increases the response of the rat to both types of shock, however, without appreciably altering the sensitivity of the uterus to histamine.
6. The similarity between the anaphylactic reaction, as well as between the uterine reaction *in vitro*, of the rat to histamine and to anaphylactic shock indicates that the anaphylactic reaction is anaphylactic in anaphylactic shock and anaphylactic in anaphylactic shock.
7. On the difference in the response of the uterus *in vitro* to both these conditions, it is not demonstrable.

BIBLIOGRAPHY

- Abel, J.J. and S. Kubota; 1919. Journ. Pharm. Exper. Therap.,
xiii, 243.
- Abel, J.J. and D.C. Macht. 1919. Journ. Pharm. Exper. Therap.,
xiv, 279.
- Adler, L. 1918. Arch. Exper. Path. & Pharm., lxxxiii, 248.
- Arthus, M.M. 1903. Compt. Rend. de Soc. Biol., lv, 817.
- Artundo, A. 1927. Metabolismo de las Ratas Suprarrenoprivas.
Universidad Nacional de Buenas Aires.
- Bally, L.H. 1929. Journ. Immun., xvii, 191, 207, 223.
- Barbour, H.G. and F.H. Rapoport. 1922. Journ. Pharm. Exper.
Therap., xviii, 407.
- Barger, G. and H.H. Dale. 1910. Journ. Physiol., xl, (proc)
" " " " " 1911 " " xxxviii.
xli, 499.
- Belding, D. and L.C. Wyman. 1926. Amer. Journ. Physiol.,
lxxvi, 191.
- Best, C.H., H.H. Dale, H.W. Dudley, W.V. Thorpe. 1927. Journ.
Physiol., lxii, 397.
- Best, C.H. and E.W. McHenry. 1929. Journ. Physiol., lxvii, 256.
" " " " " 1930. " " lxx, 349.
" " " " " 1931. Physiol. Rev., xi, 371.
- Berthelson, K.C. and P.P. Murdick. 1931. Journ. Immun., xxi, 407.
- Blair, E.W. 1922. Anat. Rec. xxiii, 9.
- Boinet, E. 1895. Soc. de Biol., 27, 1295.
" " 1896. Compt. Rend. Soc. de Biol., 364, 403, 466.
- Cannon, W.B. 1918. J.A.M.A. lxx, 611. 
" " 1923. Traumatic Shock. W.B. Appleton & Co., N.N. 1923.
" " 1931. Amer. Journ. Physiol., xcvi, 447.

- Civellari, C.A. 1926. Rev. Soc. Argent. Biol., 1, no. 6448. ✓
- " " 1927. Amer. Journ. Physiol. lxxxi, 414.
- Cow, D. 1919. Journ. Physiol., 49, 367.
- " " 1919-20. Journ. Pharm. Exper. Therap., xiv, 275.
- Dale, H.H. 1920. Bull. Johns Hopkins Hosp., xxi, 310.
- " " 1929. Lancet, ccxvi, 1179, 1233, 1285.
- Dale, H.H. and P.P. Laidlaw. 1912. Journ. Pharm. Exper. Therap.,
iv, 75, 167.
- " " " " " 1913. Journ. Pharm. Exper. Therap.,
iv, 517.
- " " " " " 1919. Journ. Physiol., lii, 355.
- Dragstedt, C.A. and E. Gebauer-Feulnegg. 1932. Proc. Exper. Biol.
Med., xxii, 891, 1084.
- " " " " " 1932. Amer. Journ.
Physiol., cii, 512, 520.
- Donaldson, H.H. 1924. The Rat. Memoirs of the Wistar Institute
of Anatomy and Biology. No. 6. Phila. 1924.
- Ebert, E.K. 1927. Ztschr. f. Immunitätsforsch. u. Exper. Therap.,
li, 78.
- Ehmer, T. and J. Hammerschmidt. 1928. Klin. Wchnschrft., vii, 931.
- Fall, F.H. 1918. Journ. Infect. Dis., xxiii, 83.
- Feldberg, W. and E. Schilf. 1930. Histamine. Julius Springer.
Berlin.
- Flashmann, D.H. 1925. Journ. Infect. Dis., xxxviii, 461.
- Friedberger, E. and Seidenhantz. 1911. Ztschr. f. Immunitäts. X
u. Exper. Therap. heft 3. ✓
- Fühner, H. 1913. Therap. Monatsh. Berlin, xxvii, 202.
- Gay, F.P. and A.R. Clark, 1924. J.A.M.A. lxxxiii, 1296.
- Galli-Valerio, B. 1910. Ztschr. f. Immunitätsforsch. u. Exper.
Therap., Jena, orig. v, 659.

Conzelmann, C.A. 1956. Rev. Soc. Hygiene. Biol., 1, no. 6448.

" " 1957. Amer. Jour. Hygiene. Biol., 1, no. 6448.

How, D. 1919. Jour. Hygiene. Biol., 1, no. 6448.

" " 1919-20. Jour. Hygiene. Biol., 1, no. 6448.

Dale, H.B. 1920. Jour. Hygiene. Biol., 1, no. 6448.

" " 1929. Lancet, xxvi, 1179, 1285.

Dale, H.B. and F.P. Leishaw. 1919. Jour. Hygiene. Biol., 1, no. 6448.

" " " " 1919. Jour. Hygiene. Biol., 1, no. 6448.

" " " " 1919. Jour. Hygiene. Biol., 1, no. 6448.

Drayton, C.A. and E. G. G. 1919. Jour. Hygiene. Biol., 1, no. 6448.

" " " " 1919. Jour. Hygiene. Biol., 1, no. 6448.

Donatien, H.B. 1924. The Nat. History of the Water Louse of Anatomy and Biology. No. 1. 1924.

" " " " 1924. Jour. Hygiene. Biol., 1, no. 6448.

Donatien, H.B. and E. G. G. 1924. Jour. Hygiene. Biol., 1, no. 6448.

Donatien, H.B. 1924. Jour. Hygiene. Biol., 1, no. 6448.

Donatien, H.B. 1924. Jour. Hygiene. Biol., 1, no. 6448.

Donatien, H.B. 1924. Jour. Hygiene. Biol., 1, no. 6448.

Donatien, H.B. 1924. Jour. Hygiene. Biol., 1, no. 6448.

Donatien, H.B. 1924. Jour. Hygiene. Biol., 1, no. 6448.

Donatien, H.B. 1924. Jour. Hygiene. Biol., 1, no. 6448.

- Greene, C.W. and C.P. Bonham. 1924. J.A.M.A., lxxxiii, 840.
- Guggenheim, M. 1912. Therap. Monatsh., xxvi, 795, 797.
- Gunn, J.A. and J.W. Gunn. 1914. Journ. Pharm. Exper. Therap.,
v, 527.
- Haendel, M. 1930. Ztschr. f. des. ges. exper. Med. lxxi, 765.
- Hanke, M.T. and K.K. Koessler. 1924. Journ. Biol. Chem., lix,
803-903.
- Harne, O.G. 1932. Amer. J. Physiol., c, 331.
- Harris, K.E. 1927. Heart, xiv, 161.
- Heller, H. and P. Holtz. 1932. Journ. Physiol., lxxiv, 132.
- Hill, J. and L. Martin. 1932. Medicine, xi, 141.
- Hunt, R. and A. Seidel. 1909. Bull. #47, Hyg. Lab. U. S. A.
- Hurwitz, S.H. and E.G. Nichols. 1930. Proc. Soc. Biol. Exper.
Med., xxviii, 139.
- Jaffe, H.L. and D. Marine. 1924. Journ. Inf. Dis., xxxv, 334.
- Jaffe, H.L. 1926. Amer. Journ. Path., v, 421.
- Jaffe, H.L. and A. Plavska. 1926. Proc. Soc. Exper. Biol. Med. ,
xxiii, 528.
- Kalk, A. 1929. Klin. Wchnschr., viii, 64.
- Kahn, A.H. and E. Starkenstein. 1911. Pflüger's Arch., cxxxiv, 181.
- Kelloway, C.H. 1930. Brit. Journ. Exper. Path., xi, 72.
- Kendall, A.I. and T.O. Shumate. 1930. Journ. Infect. Dis., xlvii,
19.
- Kennedy, W. 1925. Journ. Pharm. Exper. Therap., xxvi, 227.
- Kepinow, L. 1922. Compt. Rend. Soc. Biol., lxxxvii, 327.
- Keyes, P. and E.R. Strauser. 1926. Journ. Immun., xii, 419.
- Klinge, F. 1927. Deutsche Med. Wchnschr., liii, 1903.
- Knaus, H. and A.J. Clark. 1925. Journ. Pharm. Exper. Therap.,
xxvi, 347, and 349.

Yasane, T.W. and G.F. Forster. 1954. J.A.M.A., 154: 1541, 1542.

Yasunobu, M. 1951. J. Neuro. Neurosurg., 13: 131, 132.

Yasunobu, M. and G.F. Forster. 1954. J. Neuro. Neurosurg., 13: 131, 132.

Yasunobu, M. 1953. J. Neuro. Neurosurg., 13: 131, 132.

Yasunobu, M. and G.F. Forster. 1954. J. Neuro. Neurosurg., 13: 131, 132.

Yasunobu, M. and G.F. Forster. 1954. J. Neuro. Neurosurg., 13: 131, 132.

Yasunobu, M. and G.F. Forster. 1954. J. Neuro. Neurosurg., 13: 131, 132.

Yasunobu, M. and G.F. Forster. 1954. J. Neuro. Neurosurg., 13: 131, 132.

Yasunobu, M. and G.F. Forster. 1954. J. Neuro. Neurosurg., 13: 131, 132.

Yasunobu, M. and G.F. Forster. 1954. J. Neuro. Neurosurg., 13: 131, 132.

Yasunobu, M. and G.F. Forster. 1954. J. Neuro. Neurosurg., 13: 131, 132.

Yasunobu, M. and G.F. Forster. 1954. J. Neuro. Neurosurg., 13: 131, 132.

Yasunobu, M. and G.F. Forster. 1954. J. Neuro. Neurosurg., 13: 131, 132.

Yasunobu, M. and G.F. Forster. 1954. J. Neuro. Neurosurg., 13: 131, 132.

Yasunobu, M. and G.F. Forster. 1954. J. Neuro. Neurosurg., 13: 131, 132.

Yasunobu, M. and G.F. Forster. 1954. J. Neuro. Neurosurg., 13: 131, 132.

Yasunobu, M. and G.F. Forster. 1954. J. Neuro. Neurosurg., 13: 131, 132.

Yasunobu, M. and G.F. Forster. 1954. J. Neuro. Neurosurg., 13: 131, 132.

Yasunobu, M. and G.F. Forster. 1954. J. Neuro. Neurosurg., 13: 131, 132.

Yasunobu, M. and G.F. Forster. 1954. J. Neuro. Neurosurg., 13: 131, 132.

Yasunobu, M. and G.F. Forster. 1954. J. Neuro. Neurosurg., 13: 131, 132.

Yasunobu, M. and G.F. Forster. 1954. J. Neuro. Neurosurg., 13: 131, 132.

Yasunobu, M. and G.F. Forster. 1954. J. Neuro. Neurosurg., 13: 131, 132.

- Kramer, B. and J. Howland. 1922. Bull. Johns Hopkins Hosp.,
xxxiii, 313.
- Kuschinsky, G. 1929. Ztschr. f. d. ges. exper. Med., lxiv, 563.
- La Barre, J. 1926. Compt. Rend. Soc. Biol., xcix, 1287.
- Langley, J.S. and H.K. Anderson. 1895. Journ. Physiol. xix, 71.
- Langlois, and Charrin, 1894. Soc. Biol., 1894, March 25th.
- Lewis, J.T. 1921. Compt. Rend. Soc. Biol., lxxxv, 685, 1214.
- " " 1923. " " " " lxxxix, 1118.
- " " 1925. Amer. Journ. Physiol. lxiv, 506.
- " " and A. Torine. 1926. Rev. Soc. Argent. de Biol., 11,
459.
- Lewis, Sir Thomas. 1927. The Blood Vessels of the Skin and their
Responses. London, Shaw and Sons, Ltd.
- Longcope, W.T. 1922. Journ. Exper. Med., xxxvi, 627.
- Manwaring, W.H. and Assoc. 1917. Journ. Immun., 11, 517.
- " " " " 1921. J.A.M.A., lxxvii, 849.
- " " " " 1923. Journ. Immun., viii, 47, 121,
131, 191, 211, 217, 229, 233, 317.
- " " " " 1923. J.A.M.A. lxxx, 303.
- Marmorston-Gottesman, J. and J. Gottesman. 1928. Journ. Exper.
Med., xlvii, 503.
- Moldovan, J. and M. Zolog, 1923. Compt. Rend. Soc. Biol., lxxxix,
341, 1242.
- Molinelli, E.H. 1926. Rev. Sud. Americ. de Endocrinol., Immunol.
y Quimolog. ix, 902.
- Nikolaeff, N.M. and L.L. Goldberg. 1930. Ztschr. f. d. ges. exper.
Med., lxxiii, 475.
- Nordman, M. 1931. Physiol. Rev., xi, 41.
- Novy, F.G. and P.H. de Kruif. 1917. Journ. Infect. Dis., xx, 618,
776, 629, 657.
- Ogata, S. 1921. Journ. Pharm. Exper. Therap., xviii, 185.
- Mellanby, E. 1915. Quarterly Journ. Med., IX, 165

- Opie, E.L. 1924. Journ. Immun., ix, 231.
- Oppenheim, R. and M. Loeper. 1901. Compt. rend. Soc. de Biol.
Liii, 316, 318.
- Parker, J.T. and F. Parker. 1924. Journ. Med. Res., xlv, 263.
- Percival, G.H. and C.M. Scott. 1931. Journ. Pharm. Exper. Therap.
xliii, 563.
- Peterson, W.F. and S. Levinson, T.P. Hughes. 1923. Journ. Immun.,
viii, 349, 361, 367, 377.
- Rackemann, F.M. 1931. Clinical Allergy, particularly Asthma and
Hay Fever, McMillan & Co., N. Y.
- Reynolds, R.N. and G. Weinstein, M.H. Friedman. 1931. Amer.
Journ. Physiol., xcvi, 237.
- Rogoff, J.M. and E.E. Ecker. 1927. Amer. Journ. Physiol.,
Lxxx, 200.
- " " " J. de " 1925. Journ. Pharm. Exper. Therap.,
XXV, 243.
- Rosenau, M.J. and G.E. Anderson. 1906. Bull. 29, Hyg. Lab.
U.S.P.H.S., 57.
- Salant, W. and W.M. Parkins. 1931. Journ. Pharm. Exper. Therap.,
xlv, 315.
- Sarnowski, . 1913. Ztschr. f. d. ges. Immunitätsforsch. u.
exper. Therap., xvii, 577.
- Schelling, D.H. 1931. Journ. Biol. Chem., xcvi, 195, 215, 229.
- Schultz, W.H. 1910. Journ. Pharm. Exper. Therap., i, 549.
- Scott, W.J.M. 1923. Journ. Exper. Med., xxxviii, 543.
- " " 1924. " " " xxxix, 457.
- " " 1928. " " " xlvii, 185.
- Schmidt, G.W. and A. Stähelein. 1929. Ztschr. f. Immunitätsforsch.
u. exper. Therap., lx, 222.
- Schwartz, O. 1910. Pflüger's Arch., cxxxiv, 259.
- Seegal, B.C. and D. Khorazo. 1929. Arch. of Path., vii, 827.

- Smith, M.I. 1920. Journ. Immun., v, 250.
- Sollman, T. 1922. Physiol.Rev., ii, 479.
- Spain, W.C. and E.F. Grove, 1925. Journ. Immun., x, 433.
- Stoland, O.C. and N.P. Sherwood, R.A. Woodbury. 1931, J. Immun. XL, 5.
- Storm, van Leeuwen, W. and A. vonSzent, Gyorgyi. 1921. Journ.
Pharm. Exper. Therap., xviii, 257.
- " " " " " " vonSzent, Gyorgyi. 1922. Ibid.,
xx, 1.
- " " " " " " " " 1925. Ibid.,
xxiv, 14.
- Trommsdorf, R. 1909. Arb. Kaiserl. Gesundheitsamt. xxxii, 563.
- Vanucci, D. 1924. Do Sperimentale, lxxxviii, 23 (cit. Gay and
Clark)
- Voegtlin, C. and H.A. Dyer. 1925. Journ. Pharm. Exper. Therap.,
xxiv, 101.
- Wedgewood, P.E. and H.H. Grant, 1924. Med. Bull. Univ. Cinn. 172.
- Went, S. and C.K. Drinker. 1929. Amer. Journ. Physiol., lxxxviii,
468.
- Wells, H.G. 1929. Chemical Aspects of Immunity. Chemical Catalogue
Co., N. Y.
- Wyman, L.C. 1928. Amer. Journ. Physiol., lxxxvii, 29.
- " " 1929. " " " lxxxix, 356.
- " " 1930. " " " xciv. 579.
- Wyman, L.C. and C. tum Suden, 1932a. Ibid., xcix, 285.
- " " " " " " 1932b. Ibid., ci, 282.
- Yoshimura, K. 1910. Biochem. Ztschr., xxviii, 16.
- Zimmermann, E. 1931. Zentralbl. f. Bakt., abt. i, 122, p. 99.
- Zinsser, H. 1923. Infection and Resistance, 3rd Edit. Mac
MacMillan & Co., N. Y.
- Zunz, E. and Van Gertyden, Bernard. 1921. Compt. Rend. Soc.
Biol., lxxxiv, 287.

PLATE I

Figure 1.

- X- Normal, sensitized female. Oestrus III. Response of the uterus in vitro to the shocking dose of 1 cc horse serum.
- Y- Same preparation. Response of the uterus to a subsequent dose of 1 cc horse serum, illustrating desensitization.
- Z- Response of the excised uterus from a normal, non-sensitized rat to histamine, 1:250,000.
- V- Same preparation, histamine 1:125,000.
- W- Same preparation as in X and Y. Response of the excised uterus to histamine, 1:1,000,000.

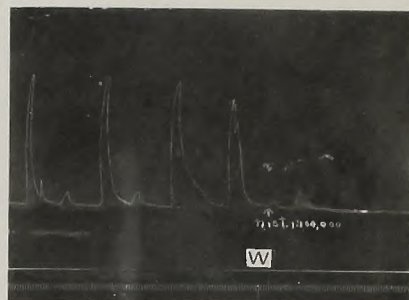
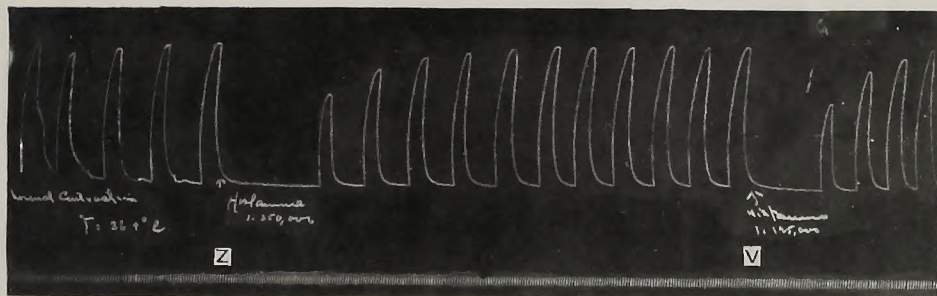
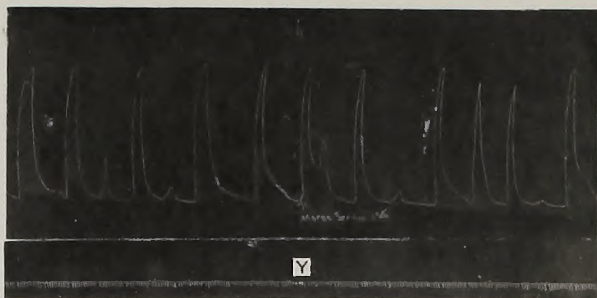
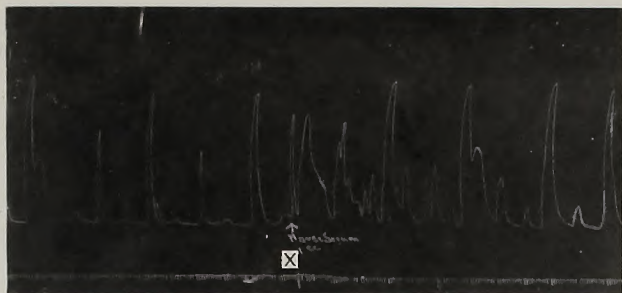
Figure 2.

- X- Sensitized female, with cortical transplants. Oestrus II. Response of the uterus in vitro to shocking dose of 0.5 cc of horse serum.
- Y- Same preparation. Response of the uterus to a subsequent dose of horse serum. 1 cc., illustrating desensitization.
- Z- Same preparation. Response of the uterus to histamine, 1:400,000.

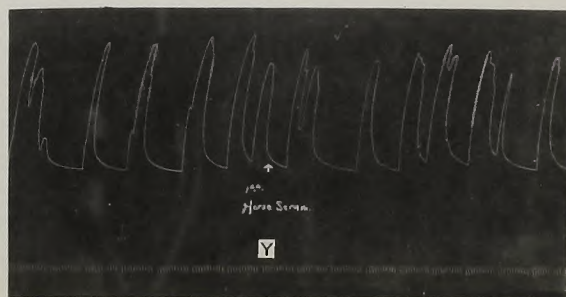
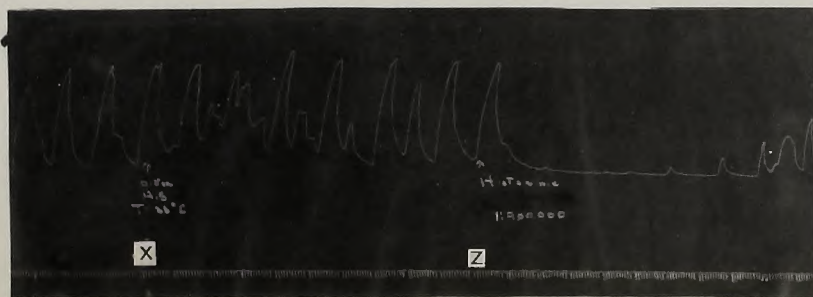
Figure 3.

- X- Sensitized, doubly adrenalectomized female. Oestrus IV. Response of the uterus in vitro to shocking dose of horse serum, 1 cc.
- Y- Same preparation. Response of the uterus to a subsequent dose of horse serum, 1 cc, illustrating desensitization.
- Z- Response of the uterus to histamine, 1:400,000, from a non-sensitized, doubly adrenalectomized female.

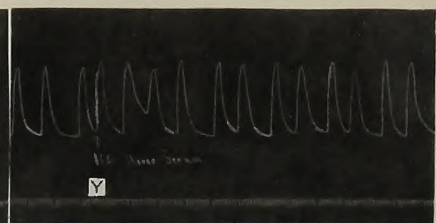
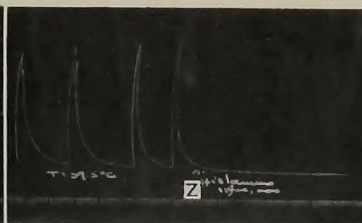
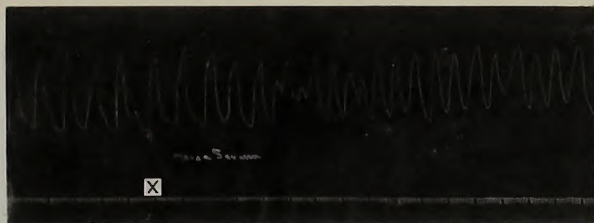
In all the figures time is recorded in 5 second intervals, and the specimens suspended in Ringer-Locke solution.



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PLATE II

Figure I.

Response of the excised uterus from a young rat to histamine, 1:1,000,000, illustrating a slight stimulating influence of histamine in small doses. Tyrode's solution. Time, 5 sec. intervals.

Figure II.

Same preparation as in Fig. I. The effect of histamine, 1:50,000, after adrenalin, 1:1,500,000. Time, 5 sec. intervals.

Figure III.

Non-sensitized, doubly adrenalectomized, 4 months old female rat. Response of the uterus in situ to direct application of histamine, 1:10,000. Time, 5 sec. intervals.

Figure I.

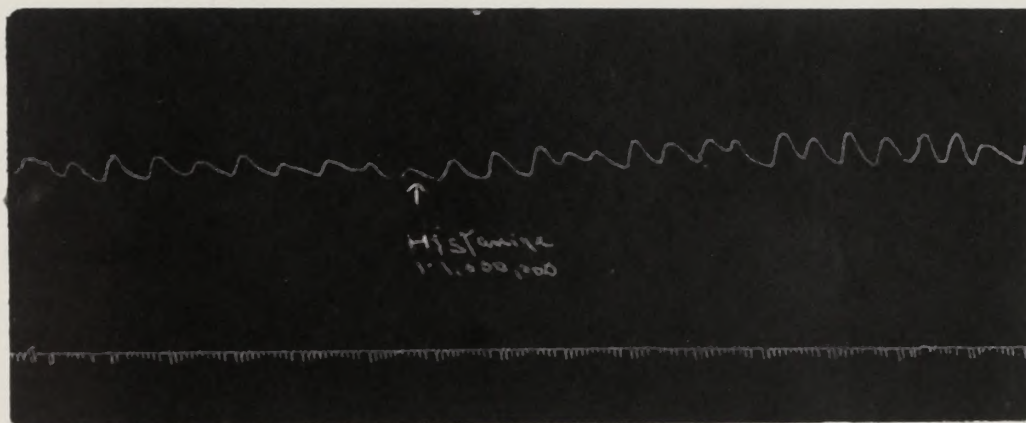
Response of the exposed uterus from a young rat to his-
tamine, 1:1,000,000, illustrating a slight stimulatory
influence of histamine in small doses. Typhoid's solution.
Time, 5 sec. intervals.

Figure II.

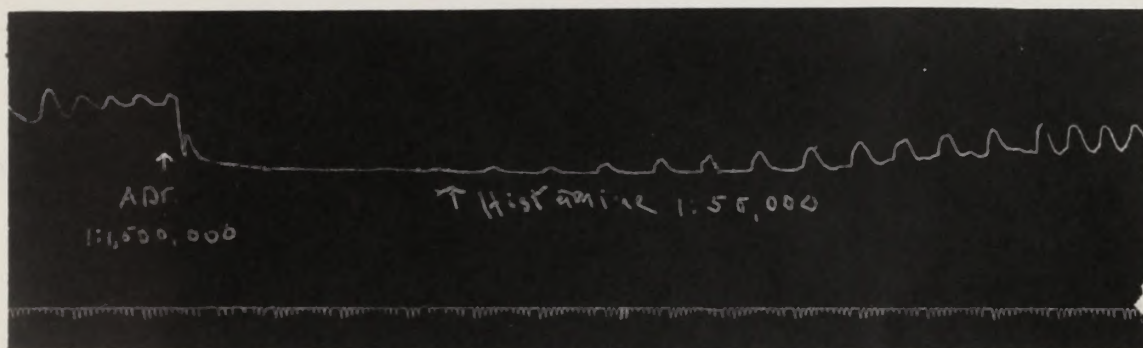
Same preparation as in Fig. I. The effect of histamine,
1:50,000, after washing, 1:1,500,000. Time, 5 sec.
intervals.

Figure III.

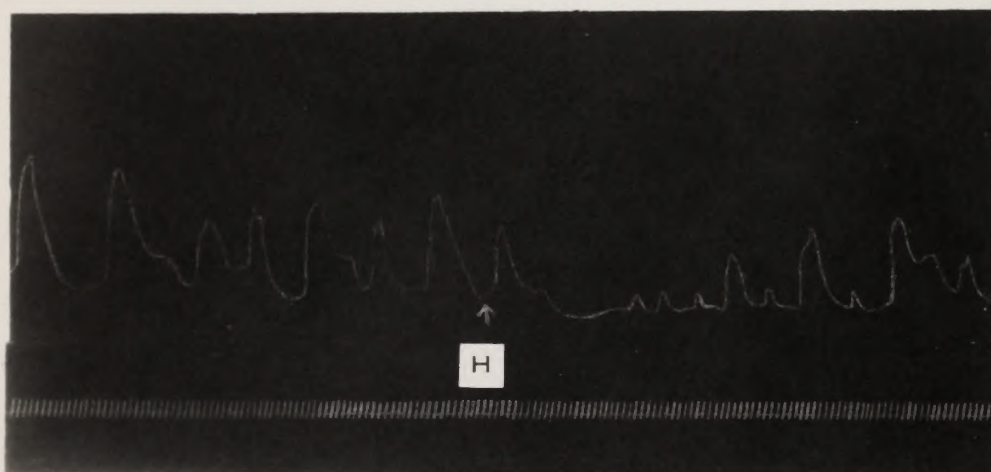
Non-sterilized, freshly administered, 4 months old
female rat. Response of the uterus in situ to direct
application of histamine, 1:10,000. Time, 5 sec. intervals.



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PLATE III

Fig. 1.

Normal, female rat, sensitized to horse serum 12 days previously. Wt., 226 gm.; oestrus, IV. A, shocking dose, 1 cc horse serum; intravenous injection at S. B, test for desensitization, injection repeated at S.

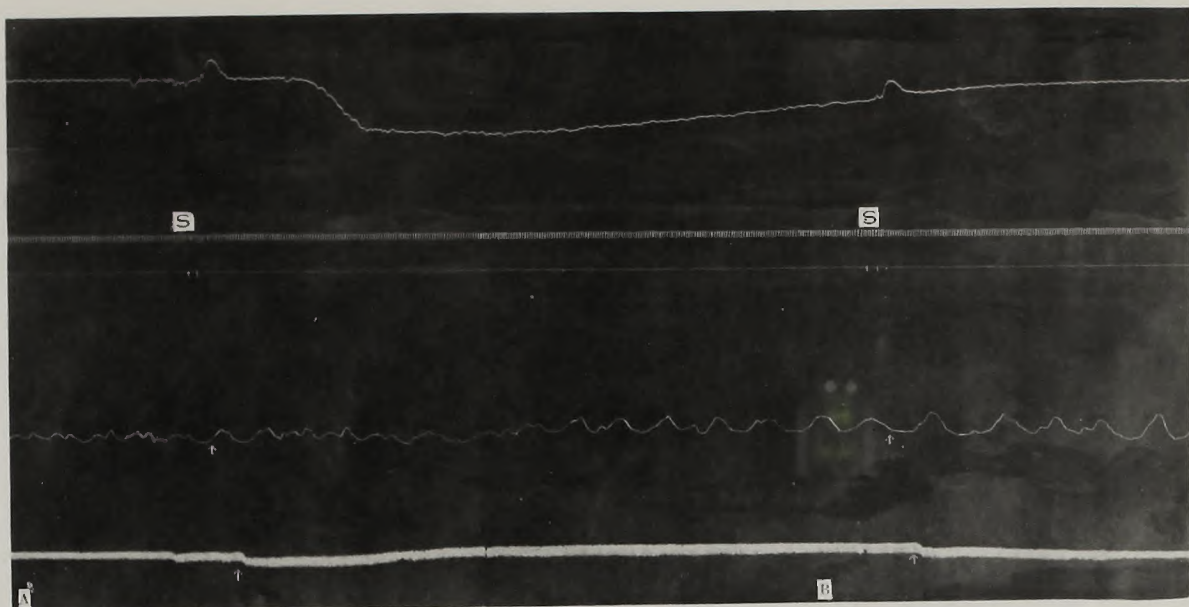
Fig. 2.

Female rat with cortical transplants, sensitized 13 days previously. Wt., 240 gm.; oestrus II. A, normal curve. B, shocking dose, 1 cc horse serum; intravenous injection at S. C, test for desensitization, injection repeated at S. Interval between B and C, 20 minutes.

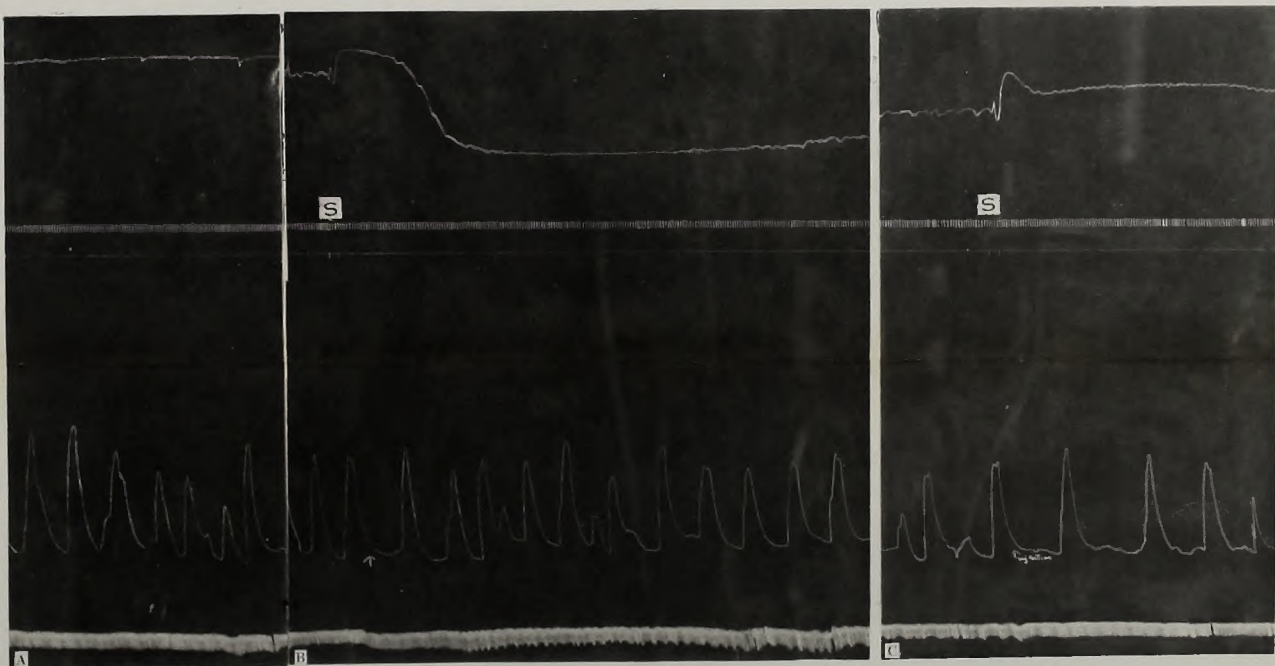
Fig. 3.

Doubly adrenalectomized rat, sensitized to horse serum, 10 days previously. Wt., 215 gm.; oestrus, II. A, shocking dose, 1 cc horse serum; intravenous injection at S. B, test for desensitization, injection repeated. Interval between A and B, 30 minutes.

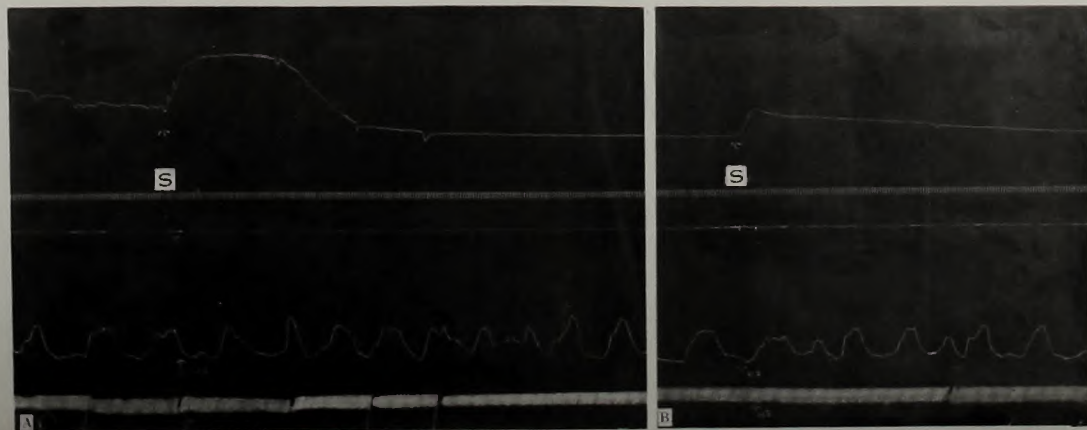
In all figures and in all the subsequent plates, time is recorded in 5 second intervals. From above down, the graphs record, blood pressure, time, injection signal, uterine activity and respiration.



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PLATE IV

Fig. 1.

Normal, female rat, sensitized 3 days previously by intraperitoneal injection of 1 cc rabbit-anti-ovalbumin serum. Wt., 180 gm; oestrus I. A, normal curve. B, shocking dose, intravenous injection of 0.04 cc 5 per cent crystalline ovalbumin at 0. C, 20 minutes after B.

Fig. 2.

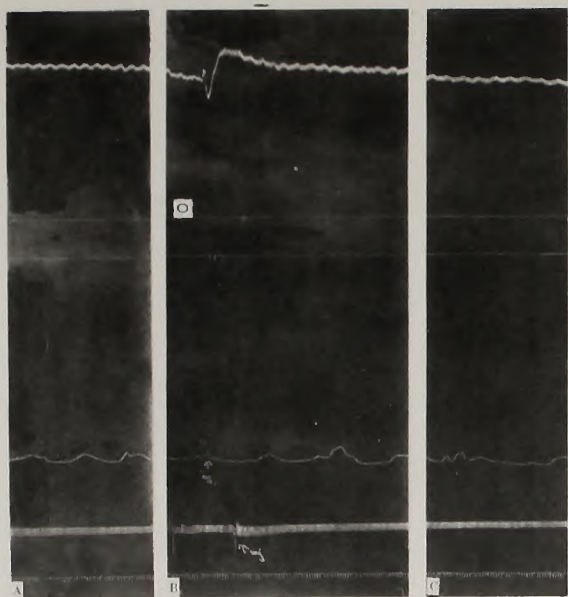
Normal, non-sensitized female rat. Wt., 200 gm; oestrus V. A, normal curve. B, intravenous injections of 0.2 cc 5 per cent crystalline ovalbumin at 0. C, intravenous injection of 0.4 cc 5 per cent crystalline ovalbumin.

Fig. 3.

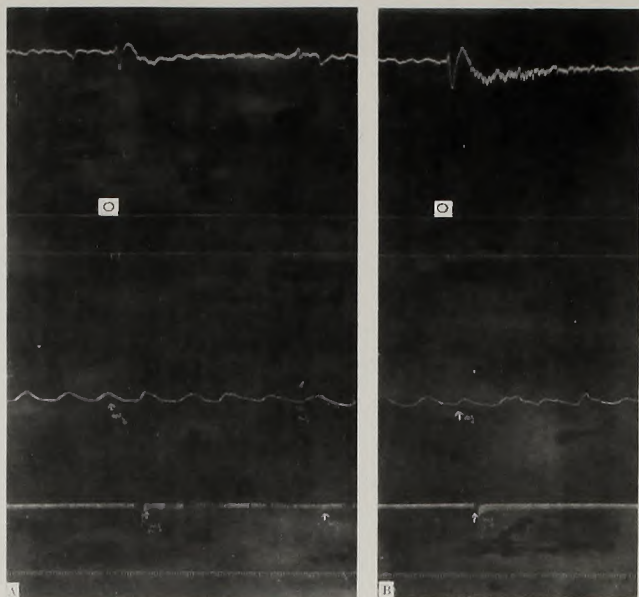
Female rat with cortical transplants, sensitized 4 days previously by intraperitoneal injection of 0.5 cc rabbit anti-ovalbumin serum. Wt. 205 gm; oestrus, II. A, normal curve. B, shocking dose at 0, intravenous injection of 1 cc 1 per cent crystalline ovalbumin. C, after 3 minutes. D, after 8 minutes. E, after 26 minutes. F, after 26 minutes, desensitization test, intravenous injection of 0.5 cc 1 per cent crystalline ovalbumin.

Fig. 4.

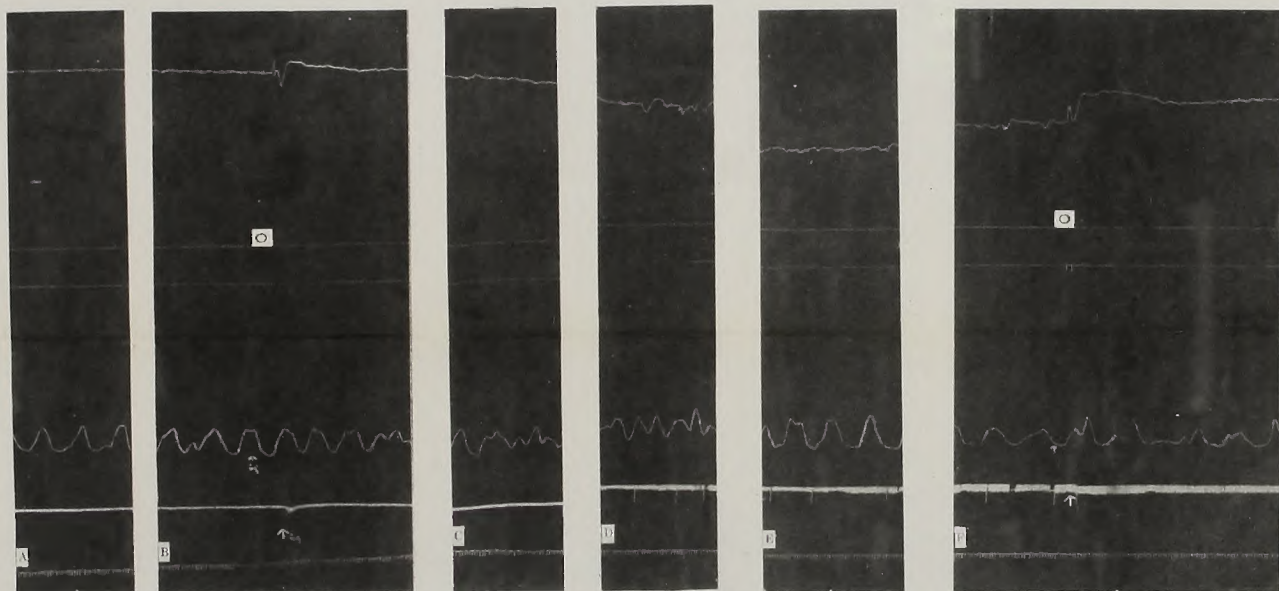
Doubly adrenalectomized, female rat, sensitized 3 days previously by intraperitoneal injection of 1 cc rabbit anti-ovalbumin serum. Wt. 190 gm; oestrus, III. A, shocking dose at 0, intravenous injection of 0.4 cc 5 per cent crystalline ovalbumin. B, after 53 minutes, desensitization test, injections repeated.



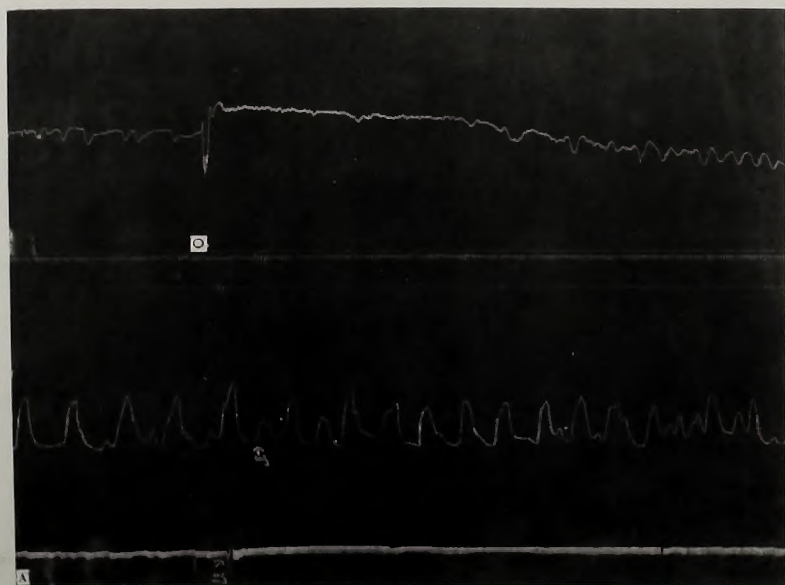
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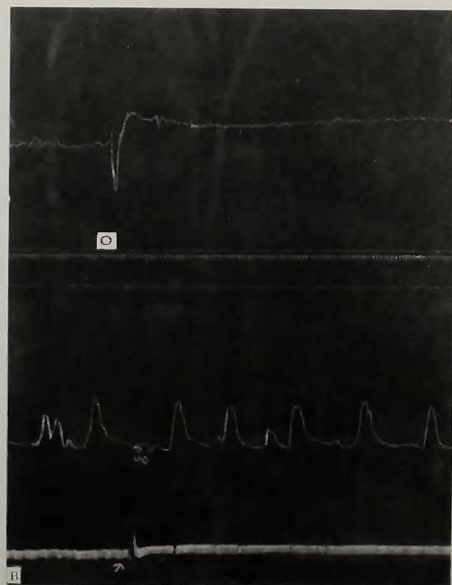


PLATE V

Fig. 1.

Doubly adrenalectomized, female rat, sensitized 13 days previously. Wt., 200 gm; oestrus I. A, normal curve, at E, application of 1:10,000 adrenalin to oozing neck incision. B, shocking dose at S, intravenous injection of 1 cc horse serum. C, 44 minutes later, death.

Fig. 2.

Doubly adrenalectomized, non-sensitized, female rat. Wt., 210 gm; oestrus III. A, normal curve. B, at S, intravenous injection of 1 cc horse serum.

Fig. 3.

Doubly adrenalectomized, female rat. Wt., 243 gm; oestrus I. At H, intravenous injection of histamine, 0.1 mgm. per 100 gm.

Fig. 4.

Normal, female rat. Wt., 195 gm; oestrus II. A, at H, intravenous injection of histamine, 0.2 mgm. per 100 gm. B, at N, induction of amyl nitrite.

Fig. 5.

Normal, non-sensitized, female rat. Wt., 190 gm; oestrus II. A, at H, intravenous injection of histamine, 0.3 mgm per 100 gms. B, at S, intravenous injection of 1 cc horse serum.

Fig. 6.

Female rat with cortical transplants. Wt., 193 gm; oestrus II. At H, intravenous injection of histamine, 0.1 mgm. per 100 gm.

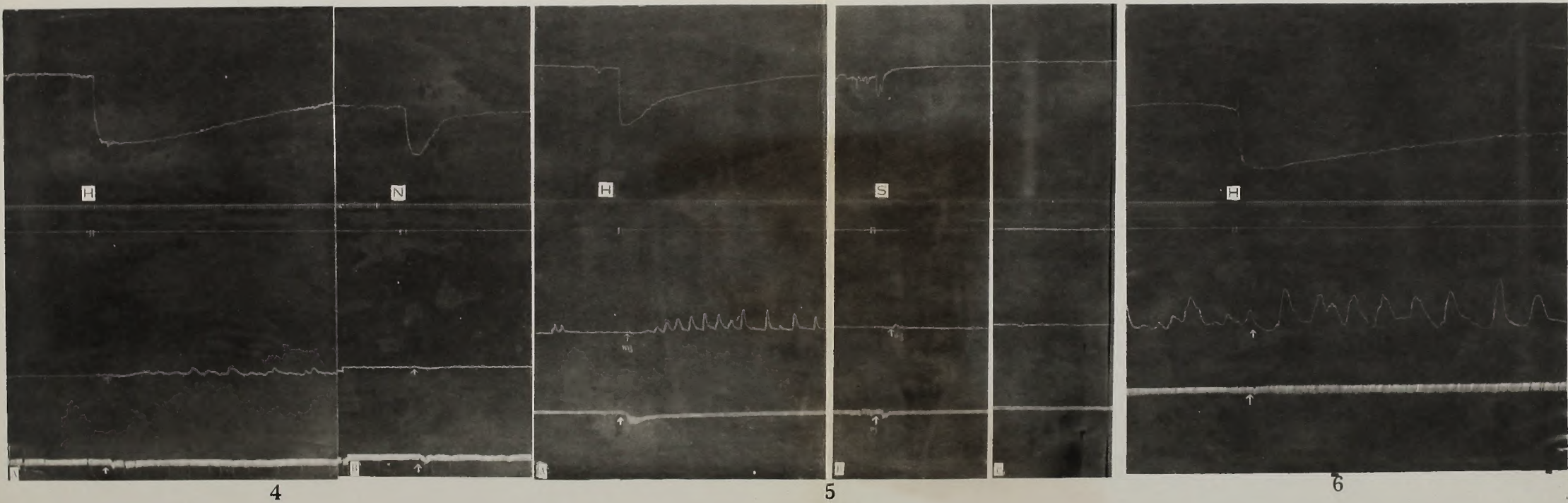
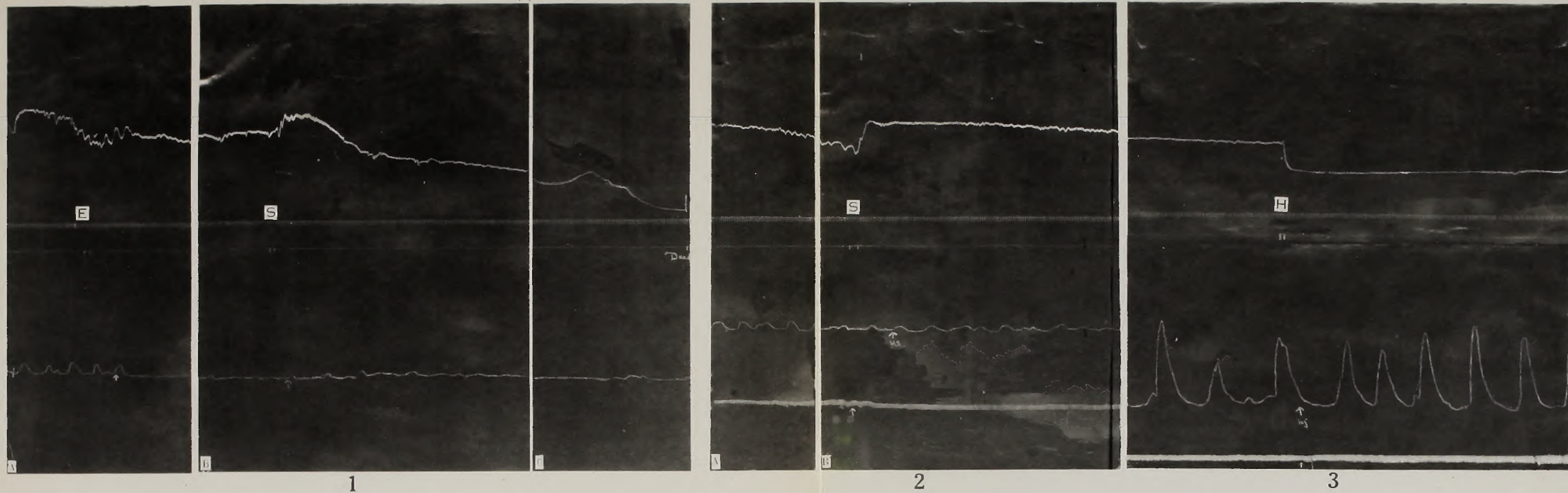
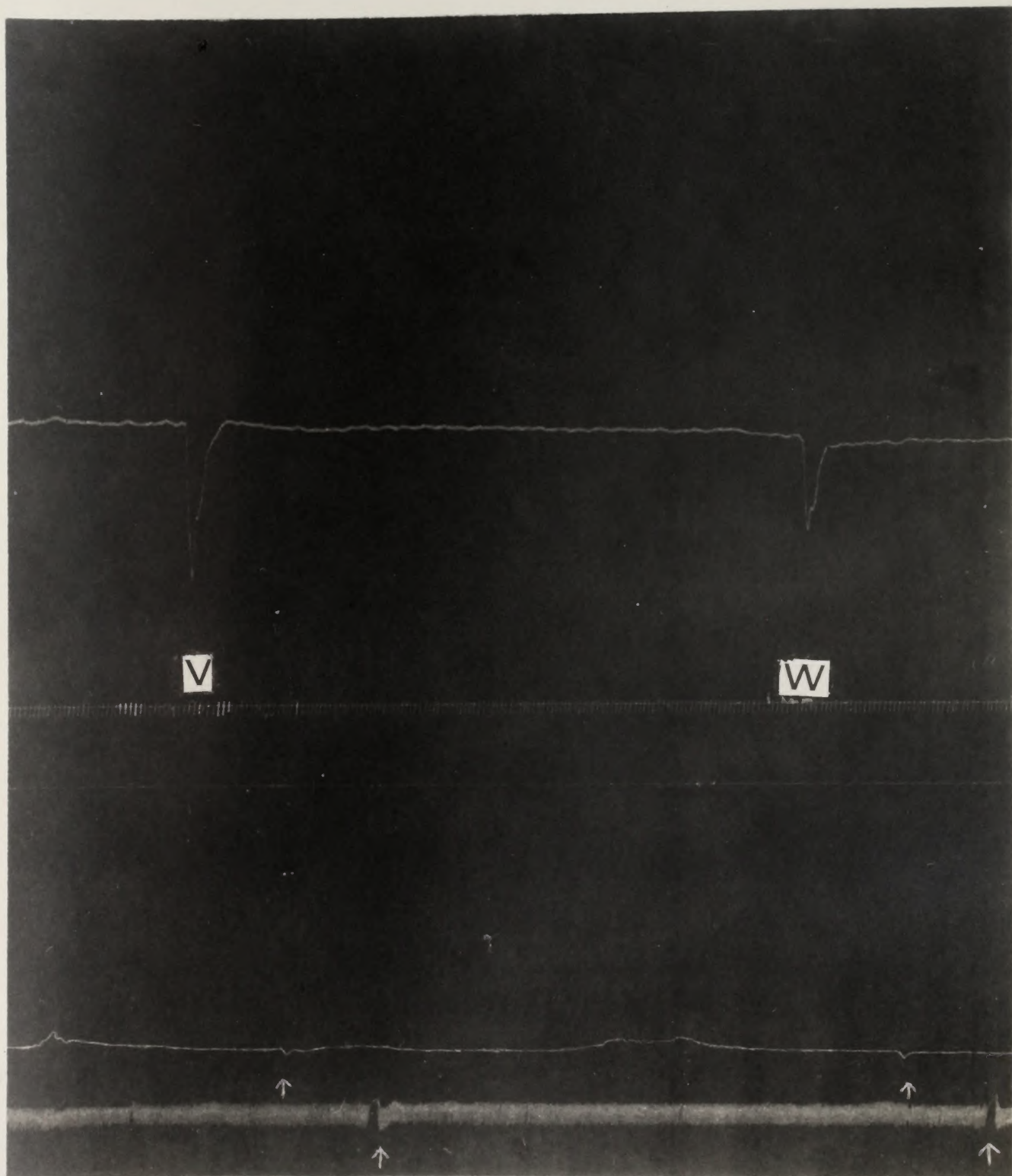


PLATE VI

Normal female rat. Wt. 192 gm; oestrus V. At V and at W,
stimulation of the right vagus nerve.

Normal female cat. No. 102 cat; section V. At V and at A.
stimulation of the right vagus nerve.



APPENDIX

A complete historical review on the matter of toxic and lethal doses of drugs and poisons for the rat would involve a search of the literature, covering every conceivable phase of pharmacological and physiological research on this and related subjects. For this reason the material compiled by Sollman in the chapter on "Animal Dosage" in his text (1928) was used as a basis for the present review. His list contains about 400 drugs, of which only 52 have been tested in the rat. A list, wherein the toxic or fatal doses for rats and other animals could be directly compared, is given in table I. From this it is apparent that about one half of the instances suggesting tolerance in the rat belong to the group of digitalis glucosides. The remainder include atropin, histamine, arsphenamine, conium, guanidine, yatren, strychnine and ephedrine. The decidedly lower resistance of the rat to epinephrine is an interesting feature.

Although the list is limited, it indicates that there is some ground for the current opinion that the rat is an extremely tolerant animal,, topping the list of species in nearly every case, but in only a few instances differing widely in tolerance from other animals in the matter of dosage. The mouse, a close relative of the rat, shows a similar behavior; hence a comparison of the two should be discounted. It may be noted at this point that Gunn (1923) states that the minimal

lethal dose of heavy metals, quinine, phenol, etc., is commonly accepted as about the same for all animals.

According to Hausmann (1907) the earliest axiom in medicine is that of acquired tolerance to drugs in man and animals. This statement may be possible extended to include congenital tolerance. Without doubt one of the earliest experiments demonstrating the natural resistance of the rat was performed in 1856, when Vulpian (according to Sollman, 1928, reference not given), showed that the rat as well as the toad was extremely insensitive to the digitalis poisons. According to Gunn (1923), Vulpian was the first to investigate the reaction of the toad to its own venom. He concluded that the venom was similar in action to digitalis, to which the toad was equally immune. From this time on, these and related problems were frequently pursued, and it was established that the same relationship held for the other members of the digitalis group (squill, strophanthin, ouabaine.) In 1909 Hatcher, in his study on the excretion and destruction of strophanthin, said that the rat was known to be nearly 1000 times as tolerant as the dog. In 1909 he ascertained the M.L.D. for ouabaine to be more than 100 mgm. per kilo of rat. In 1913 Gunn confirmed these findings and determined the fatal dose (subcutaneous). The rat required 30 times the rabbit dose, and this same difference obtained in the concentration necessary to arrest the isolated hearts of the two species. For this reason he concluded that the rat heart tissue itself was relatively in-

susceptible to the action of the glucosides.

The use of belladonna for its mydriatic and poisonous effects is recorded by historians on the Renaissance, but they made no comment on the discovery that rabbits could eat the greens of this plant with impunity. Yet this circumstance led to experiments of this nature (Heckel, 1875), and is in some measure responsible for the rabbit's reputation for tolerance to atropin. Richet in 1895 stated that the rat, which could withstand a dose of 1.0 gm. of atropin sulphate, should be classed with the rabbit. In 1912 Clark, studying the factors determining tolerance, found that the subcutaneous M.L.D. for the rat varied with age, being about 0.5 gm-kilo in the young and 1.0 gm per.kilo in the adult. He pointed out that this change with age would account for the discrepancies in the different dosages reported. Willberg (1914), upon re-investigating the subject in several species, found that the rat and fowl were the most resistant, the average M.L.D. for the rat being 0.75 gm-kilo while that for the rabbit was 0.5 gm per kilo. Sollman, even later (1928), still lists the fatal dose in his text as 2.5 gm per kilo. Voegtlin and Dyer (1925) commenting on Willberg's figures decided that the differences between the various species were not significant of unusual resistance in the rat. From their study of rat resistance they concluded that tolerance was only evidenced to those drugs which acted upon the smooth muscle or capillary endothelium, and not to those acting primarily on the nervous

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tissue, e.g. morphine, strychnine, atropin, etc.

Ephedrine, an alkaloid obtained from the Chinese plant, Ma Huang, has recently claimed attention in therapeutics. It seems that K. K. Chen is the only investigator who has made any study of its comparative toxicity, which is quite low for most animals. The M.L.D., in milligrams per kilo, he found to be as follows: frog, 530; mouse, 200; rat 140; rabbit, 66; cat, 75; dog, 75. Of the warm blooded animals, the mouse and the rat head the series but the required dose is only twice that for the others, which is not a significant variation. According to Chen, the figures indicate rather an "interesting" susceptibility in the rabbit.

In all the research done on guanidine, since Koch in 1912 pointed out the similarity between guanidine poisoning and parathyroid tetany, and Paton and Findlay's extensive work elaborated this suggestion in 1917, only one paper was found which contained some information about the comparative toxicity of this drug. The figures given by Klinger (1921) for the M.L.D. for rats and cats by intraperitoneal injections in grams per kilo, were respectively 0.15-0.13 and 0.1-0.2. The cat dose given by Sollman, 0.01-0.02 gm, is undoubtedly a typographical error since he referred to Klinger as the source of his information.

In 1864 Claude Bernard compared the narcotic power of morphine and allied alkaloids in many of the common laboratory animals. Although he stated that the rat was most easy to

narcotize, no reference to susceptibility of the different animals could be discovered in his paper. In Wood's textbook, "Materia Medica and Toxicology" (1880), the past literature on the physiological action of morphine in different animals was surveyed. In the opinion of this author the size of an effective dose correlated with the development of the cerebral organization, man and frogs occupying the extremes of the series, with every gradation in between. This would indicate that so far no true case of congenital tolerance had been encountered, although Weir Mitchell in 1868 claimed that birds were highly resistant. In Sollman's latest text (1931), man is said to be as sensitive as the cat and the dog; the rat, 15 times as resistant as man; goats, pigs, rabbits and guinea pigs about 100 times as resistant. According to the discussion in Git-chen's text, goats and pigs are almost immune, and the following fatal hypodermic doses were given in grams per kilo; cat, 0.04; horse, 0.007; cattle, 0.015; dog, 0.12; rabbit, 0.35; guinea pig 0.4; and rat and mouse 0.4. Hunt, in 1908, in his work on the effect of restricted diet on drug tolerance, obtained the following: rat 0.20-0.42 gm-kilo; mice 0.18-0.45 gm-kilo; guinea pig 0.6-0.75 gm-kilo. This work is frequently referred to. It is also interesting to note in relation to this subject that he remarked that it is "folly" to determine fatal doses very accurately, for as his work showed, susceptibility can be markedly altered by diet in addition to the individual variation so often met with. It is evident from

the figures given that no marked tolerance to morphine is exhibited by the rat. As for papaverine, Wood stated that all mammals tolerate fairly large doses.

Schwarze's use of the term "remarkable resistance" of the rat in regard to strychnine suggests that previous to his work others have entertained such a conception. Using the method of Hatcher and Eggleston (1917), he estimated that 20.8 percent to 33.3 percent of this drug was eliminated within the first hour. Consequently, this rapid excretion accounted for the ability of the rat to tolerate consecutive injections of strychnine. Hatcher and Eggleston had previously shown that that the guinea pig eliminated from 18 percent to 57 percent per hour; while the highest rate obtained for the rat and dog were respectively 25 percent and 19 percent. These facts are in harmony with the subcutaneous minimal lethal dosage as given by Schwarze; cat, 0.32; dog, 0.38; rabbit, 0.4; guinea pig, 3.4; rat, 2.3; mouse, 0.78; (all mgm. per kilo gram body weight). Schwarze also demonstrated that the young of most species are more resistant to this drug immediately after birth. This tolerance gradually diminished and finally reached a level at maturity, but in the case of rats and guinea pigs, after an initial decline, tolerance was re-acquired and remained at a high level. Since the rat and guinea pig lethal dose is about 10 times that for other species, some degree of tolerance may be attributed to them.

The extensive use of arsenicals in spirochaete and

The first thing I noticed when I stepped out of the car was the cold air. It was a relief after the warmth of the car. I looked around and saw a few people walking on the sidewalk. They were all dressed in winter clothes. I felt a little out of place.

I walked towards the entrance of the building. The door was open and I went inside. The interior was dimly lit. I saw a receptionist sitting at a desk. She looked up at me and smiled. I introduced myself and she showed me to my room.

I went to my room and took a shower. The water was hot and I felt relaxed. I then went to bed and fell asleep. The room was comfortable and I was happy to be there.

The next morning I woke up early. I got out of bed and went to the bathroom. I looked in the mirror and saw a tired man. I took a shower and got dressed. I then went to the breakfast room.

I sat at a table and ate my breakfast. The food was good and I enjoyed it. I then went back to my room and packed my bag. I was ready to go home.

I took a taxi to the airport. The driver was friendly and the ride was smooth. I arrived at the airport and saw my family. They were all happy to see me and I was happy to see them.

I went home and everything was the same. I was happy to be back and I enjoyed my trip. I then went to bed and fell asleep.

The next morning I woke up and went to work. I was tired but I knew I had to go. I worked hard and finished my day. I then went home and relaxed.

I was happy to be back and I enjoyed my trip. I then went to bed and fell asleep.

trypanosome infections has led to considerable study of the comparative toxicities of arsenic and its numerous organic derivatives. There are only a few instances where the figures for the fatal dose for different animals can be compared. Even in those cases where the same technique and procedure has been employed, disagreements occur. For example, in Sollman's text the lethal dose by vein for a rabbit of a 5 percent solution of arsphenamine, is given as 0.004 gm. per kilo whereas Gitchens quotes the dose as 0.112 per 0.125 gm. per kilo by the same method. Kochmann stated (1913) that 0.2 gm. per kilo was fatal. However, the concentration was not given by the latter author. According to Gitchens, there is much confusion about the relative toxicities of the arsenicals, which is due to many factors, such as marked individual variation, speed of injection, concentration of the substance, allied toxic products, and viscosity changes. Heyl (1923) stated that, owing to the instability of the preparation, impurities developed on storage and were responsible for the variations in the toxicity of different batches of arsphenamine and the derivatives. Voegtlin and Leonard (1923) showed, furthermore, that the toxicity factor paralleled the viscosity changes, which developed on ageing. The M.L.D. for rats varied from 4 mgm. to 20 mgm. per kilo under these circumstances. In 1923 they also showed that there was a great difference among the various organic arsenic compounds, especially trivalent and pentavalent, for where 1000 mgm. of tryparsamid would be

necessary to kill a rabbit, only 20 mgm. of arsenoxide was needed. Figures for the rat and rabbit M.L.D. obtained from their papers were as follows: rat, 10 cc. of 0.1 M. arsenoxide per kilo; rabbit, 1 cc. of 0.1 M. arsenoxide per kilo. They claimed that there was no important species difference in the matter of toxicity. According to Willberg (1913) this is also true of the inorganic arsenic compounds when given subcutaneously or intravenously

On the latter subject, Schwarz (1922) and Hammett and Nowry (1922) have obtained some interesting data from their study of arsenic poisoning in rats. The M.L.D. of potassium arsenite in mgm. per kilo were as follows: rabbit, 10, guinea pig, 9, dog 7, rat, 5 to 6, mouse, 15. Since the latter authors were able to demonstrate a difference in the fatal subcutaneous dose for young and adult rats (11 mgm. and 8 mgm. per kilo respectively, of a 0.2 percent solution of potassium arsenite), they believed that the change was due to a difference in the metabolic rate in the old and in the young animal. They estimated that the rat is 2.4 times as tolerant as man, and further that the metabolic rate in the rat, determined by the urinary nitrogen excretion, exceeded that of man proportionately, all of which, taken together with the other findings, nicely supported their contention.

In 1923 Schwarze discussed the reason for much of the prevailing confusion on the relative toxicity of arsenic in man and animals in his paper entitled, "So-called Habituation

to Arsenic". In the first place he demonstrated that habituation did not obtain in animals, confirming the negative results of Brouardel (1897) and Morishima (1900). The more recent work of Sollman (1921) supported this fact, for he found that very small doses of arsenic were not beneficial to rats, the decrease in growth rate indicating chronic intoxication.

Another reason why animals and man are able to survive large or increasing amount of orally administered arsenic, i.e. arsenic oxide, according to Schwarze, is not species tolerance but the physical state of the substance given, namely the size and ease of solubility of the particles ingested. He found that there was more variation between species with orally administered solution, as the oxide, than with the subcutaneously or intravenously administered doses; and that with undissolved particles the differences were greatly increased as well as the size of the fatal dose. In fowls he found a slight exception to the rule, for here large particles were subjected to grinding in the gizzard. Consequently the increase in the fatal dose did not run parallel to the increasing size of the particles. For the rat 75 mgm. per kilo of dissolved AsO was fatal as compared to 15 mgm for the rabbit. If the substance was given undissolved, the rat then required 500 mgm and the rabbit 200 mgm.

From the above discussion it would seem that the rat and mouse fatal doses are the highest for the mammalian series and may indicate a slight degree of tolerance, contrary to the

opinion of Voegtlin and Dyer.

In connection with the above resumé, the peculiarities of the rat with respect to the hormones, parathyroid extract and pituitrin, should be mentioned. In 1926 Greenwald and Gross stated that the rat and rabbit tolerated phenomenal overdosages of parathyroid extract. They attributed this ability to an effective excretory mechanism for calcium. Burns (1927) confirmed this fact in a measure, for he found that there was no retardation of body or bone growth after prolonged feeding with the extract.

As regards pituitrin, both Voegtlin and Dyer (1925) and Knaus and Clark (1925) found that the uterine muscle strip of the rat was much less sensitive than that of other animals. Knaus (1925) stated that it took a concentration of 1:1,000,000 of fresh pituitary extract to obtain a visible contraction of the rat isolated uterus, whereas a concentration of 1:10,000,000 produced a marked effect on the guinea pig muscle. In one record published, a concentration of 1:250,000 did not show a very pronounced response. Voegtlin and Dyer stated that a concentration of 1:50,000 pituitrin tartrate or standard pituitary was necessary to elicit a good contraction, whereas Smith and McKloskey had conclusively shown that a dilution of 1:2,000,000 to 1:10,000,000 was effective on the uterine strips of other species. Voegtlin and Dyer also obtained relaxation of the isolated jejunum of the rat with a concentration of 1:50,000 of the same preparation instead of contraction, which is the usual

action seen in other animals.

The data on histamine and various types of shock for the rat are included in the main body of this paper.

Bacterial Toxins and Microorganisms. The pathogenesis of the various microorganisms for the lower animals is briefly discussed in all texts on bacteriology, from which one may readily note that the rat, as a general rule, falls into the insusceptible or relatively insusceptible group. Comparatively speaking, the broad statement that the rat is resistant to most organisms (Zinsser) is true, for the rat is more or less immune to infections with such organisms as staphylococcus, pneumococcus, glanders bacillus anthrax bacillus, tubercle bacillus (avian and bovine) and diphtheria bacillus, all of which can be transmitted to or produced in other laboratory animals. The immunity of the rat to anthrax was reported by Von Behring in 1881. Steinbach (1932) credits Colin (1873) as being the first to disclose the similar behavior of the rat to tuberculosis. Subsequently he was confirmed by many others, (Koch, 1894; Straus, 1895; Galli-Valerio, 1917; Glyne and Page, 1929; Boquet and Magee, 1921; Goldenberg, 1929; Ornstein and Steinbach, 1925). The striking immunity of the rat to diphtheria toxin has been recognized for many years. In 1921, Coca, Baughman and Russell were able to show that the fatal dose of diphtheria toxin for the rat was 4000 guinea pig M.L.D.s. Apparently up to this time the general opinion was that, since the rat survived at least 100 guinea

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pig M.L.D.s, it would not succumb to the toxin. Although there is probably more species specificity and variation in the susceptibility of animals to bacterial invasion than to the toxicity of drugs, the data on the rat indicate a more consistent, exceptional behavior than is the case with the average laboratory animals.

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TABLE I.

Data from Sollman's Experimental Pharmacology, Animal Dosage,
Comparative M.L.D.s for Rat and Other Species.

Drug	Animal	Route	M.L.D. gm or mgm. per kilogram.
Amytal	rat	hypoder.	0.1 gm
	rabbit	"	0.11 gm
Benzyl Alcohol	cat & mouse	"	1.0 cc
	guinea pig	"	1-2.5 cc
	rat	"	1-3.0 cc
Arsphenamine	rat	vein	0.1 gm
	rabbit	"	0.004 gm
	dog	"	0.01-0.02 gm
Mon or disodium Arsphenamine	rat	"	0.1 gm
	rabbit	"	0.1 gm
Sodium Barbitol	rat and cat	hypoder.	0.3-0.35 gm
	rabbit	"	0.4 gm
	dog	"	0.45 gm
Barium Chloride	rat, rabbit	Gastric	0.35-0.5 gm
	cat, dog	"	0.09 gm
Conium	rat	hypoder.	40.0 gm
	guinea pig	"	0.5 gm
Convalleria	rat	"	32.0 gm
	guinea pig	"	0.08 gm
	frog	"	0.18 gm
Diethylparaphenylene- diamine	rat	"	0.1 gm
	rabbit	"	0.25 gm
	guinea pig	"	0.2 gm
Dimethylparaphenylene- diamine	rat	"	0.05 gm
	rabbit	"	0.06 gm
	guinea pig	"	0.1 gm
Digitalis	rat		see text.
	rabbit		
	cat		
	dog		
Ephedrine sulphate	rat	vein	0.135-0.14 gm
	rabbit, cat, dog	"	0.066-0.07 gm
	mouse	"	0.16-0.2 gm

(xiv)

Epinephrine	rat	vein	0.005-0.05 mgm
	dog	"	0.1-0.25 mgm
	cat	"	0.5-0.8 mgm
	rabbit	"	0.2-0.6 mgm
	guinea pig	"	0.1-0.2 mgm
Gelsenium	rat	hypoder.	2.2 gm
	guinea pig	"	1.75 gm
Guanidine sulphate	rat	--	0.15-0.2 gm
	cat	--	0.02- gm
Helleborus niger	rat	hypoder.	20.0 gm
	frog	"	0.3 gm
	guinea pig	"	0.2 gm
Histamine	rat	hypoder.	0.9 gm
	mouse	"	0.75 gm
	rabbit	"	0.001-0.002 gm
Magnesium sulphate	rat tolerates intraperitoneal injections of 5 cc 1 percent sol. per hour		
Morphine HCl or SO ₄	rat	hypoder.	0.42 gm
	cat	"	0.04-0.08 gm
	dog	"	0.04 gm
	guinea pig	"	0.7 gm
	rabbit	"	0.2-0.32 gm
	mouse	"	0.6 gm
Ouabain (g-strophanthin)	rat	vein	100 times cat (Hatcher)
	rabbit	"	0.2 mgm
	cat	"	0.1 mgm
	dog	"	0.125-0.165 mgm
Papaverine	rat	hypoder.	0.3 gm symptoms only
	guinea pig	"	0.1-0.2 gm " "
Rivanol	rat	vein	40.0-45.0 mgm
	mouse	"	17.0-35.0mgm
Scilla	rat	hypoder.	20.0 gms
	guinea pig	"	0.4 gm
	frog	"	0.6 gm
Strophanthin	rat	see text.	
	rabbit		
Strychnine	rat	hypoder.	3.0-3.5 mgm
	guinea pig	"	3.0-4.4 mgm
	rabbit	"	0.5-0.6 mgm
	dog	"	0.3-0.42 mgm
	cat	"	0.3-0.42 mgm
	mouse	"	0.76-2.0 mgm
	fowl	"	2.0 mgm

Uranium salts	rat	hypoder.	0.41 mgm
	dog	"	1.66 mgm
	cat 0.41	"	0.41 mgm
	rabbits	"	0.83 mgm
	birds	"	40.0-44.0 mgm
Yatren	rat	--	0.6 gm
	rabbit	--	0.4 gm
	cat	--	0.36 gm
	mouse	--	0.63 gm

- Bernard, Glaude. 1864. Arch. Generales, iv.455, series 6c.
- Burns, C.M. 1929. Biochem. Journ., **xxiii** , 853.
- Bouradel, 1897. (see, Schwarze, 1923)
- Chen, K. K. 1926. Journ. Pharm. Exper. Therap., xxvii, 61.
- Clark, A. J. 1912. Quart. Journ. Exper. Physiol., v. 385.
- " 1913. Journ. Pharm. Exper. Therap., iv, 399.
- " 1913. " " " " v, 215.
- Coca, A.C. and W.H. Baughman, E.T. Russell. 1921.
Journ. Immun., vi, 387.
- Gunn, J. A. 1913. Journ. Pharm. Exper. Therap., iv, 225.
- " 1923. Physiol. Rev., iii, 41.
- Hatcher, R. A. 1909. Amer. Journ. Physiol., xxiii, 303.
- Hatcher, R. A., and C. Eggleston. 1917. Journ. Pharm. Exper.
Therap. x, 281.
- Hausman, W. 1907. Ergebnisse der Physiologie.
- Hammet, F.S. and J. E. Nowry. 1922, Journ. Pharm. Exper. Therap.
xix, 331
- Heckel, C. R. 1875. Acad. de Paris. lxxx, 1608.
- Heyl, F. W. 1923. Journ. Amer. Pharm. Assoc., xi, 214.
- Hunt, R. 1910. Bull. 69, Treas. Dept. Public Health and Mar.
Serv. U. S. Hyg. Lab.
- Greenwald, I. and J. Gross. 1926, Journ. Biol. Chem., lxviii, 325.
- Koch, 1912. Journ. Biol. Chem., xii, 313.
- Klinger, R. 1921. Arch. Exper. Pharm. u. Path., xc, 129.
- Knaus, H. and A. J. Clark. 1925. Journ. Pharm. Exper. Therap.,
xxvi, 347

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- Paton, D. N. and L. Findlay. 1916. *Quart. Journ. Exper. Physiol.*,
x, 203, 377.
- Morishima, 1900. (see Schwarze, 1923.)
- Richet, 1895. *Dict. de Physiol.*, 822.
- Schwarze, E. W. 1922. *Journ. Pharm. Exper. Therap.*, xix, 49, 273.
" " 1923. " " " " xx, 181.
- Smith, M. I. and W. T. McCloskey. 1923. *Pub. Health Reports.*,
xxxviii, 493.
- Steinbach, M. M. 1932. *Amer. Rev. Tuberculosis*. Xxvi, 52.
- Voegtlin, C. and Lennard. 1923. *Public Health Reports.*, xxxviii,
1003 and 1880.
- von Behring. 1888. *Zentralbl. f. Klin. Med.*, s., 38.
- Voegtlin, C. and H. A. Dyer. 1925. *Journ. Pharm. Exper. Therap.*,
xxiv, 101.
- Willberg, M. H. 1914. *Biochem. Ztschr.*, lxvi, 389.
- Sollmann, T. 1928. *Manual of Pharmacology*. W.B. Saunders & Co.,
Philadelphia and London, p. 513.
- Sollmann, T. and P. J. Manzlik. 1929. *Experimental Pharmacology*.
W. B. Saunders & Co., Phila. London.
- Wood, H. C. 1880. 1928. *Therapeutics, Materia Medica, and Toxicology*. J. B. Lippincott & Co., Phila.
- Gitchens, C. T. 1928. *Pharmacotherapeutics, Materia Medica, and Toxicology*. Appleton & Co., N. Y. & London.
- Zinsser, H. 1931. *Resistance to Infection*. Macmillan Co., N. Y.
- Sollmann, T. 1921. *Journ. Pharm. Exper. Therap.*, xviii, 43.
- Mitchell, Weir. 1868. (see Wood, 1880).

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IN THE DEPARTMENT OF THE HISTORY OF ARTS
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JANUARY 1911
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CHICAGO, ILLINOIS
JANUARY 1911



Autobiographical Data.

Born in San Francisco, California, of
Mr. and Mrs. Otto tum Suden(nee' Medeau) July
14th, 1900; educated at Miss West's and Miss Burke's
Schools, Mills College, 1917-1918, University of
California, 1919-1922, A.B.; University of Columbia,
1927-1928, A.M.; Simmons College, 1928; Boston
University, 1929-1930. Occupation, Research Fellow
of the Evans Memorial Hospital.



Investigative Data.

Born in San Francisco, California, of
Mr. and Mrs. Otto Paul (nee) Hansen - Mrs.
1898, 1900; educated at West Coast and other schools
schools, Santa Clara, 1917-1919, University of
California, 1921-1923, A.B.; University of California,
1923-1925, A.M.; Stanford College, 1925; Boston
University, 1925-1930. Occupation, Research Fellow
at the University of California.

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